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DENHAM, H. J.

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REFERENCE TO THE COTTON PLANT**

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HUMPHREY JOHN DENHAM, M.A.(Oxon.), F.R.M.S.

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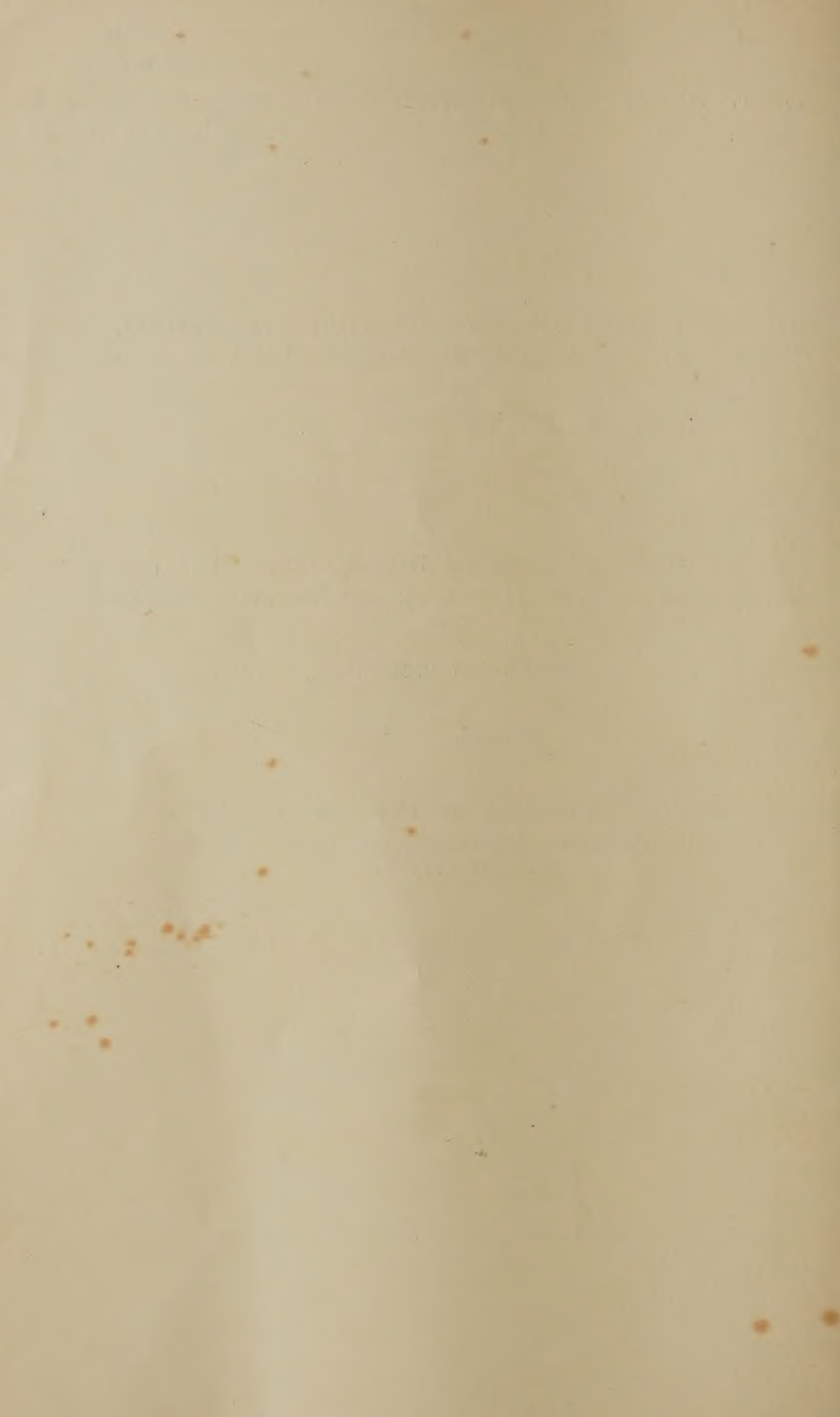
XXI.—THE CYTOLOGY OF THE COTTON PLANT
**ii.—CHROMOSOME NUMBERS OF OLD AND NEW
WORLD COTTONS**

BY

HUMPHREY JOHN DENHAM, M.A.(Oxon.), F.R.M.S.

The British Cotton Industry Research Association
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XIX.—AN INTRODUCTION TO CYTOLOGY WITH SPECIAL REFERENCE TO THE COTTON PLANT

By HUMPHREY JOHN DENHAM, M.A.(OXON.), F.R.M.S.

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INTRODUCTION

The following two papers, "The Cytology of the Cotton Plant," Parts I. and II., contain an account of some researches carried out at the Shirley Institute into the cytology of the cotton plant, and the chromosome numbers of the more important species of cotton; and it has been suggested that a brief and simplified account of the meaning and purpose of cytology (with a summary of these papers) might be of more general interest, since cytology is an extremely specialised branch of science, and, like other specialised subjects, has acquired a vocabulary of its own. The processes with which cytology deals are in themselves complex, and their nature and meaning has not, in many cases, been conclusively established, with the inevitable consequence of divergent interpretations of the same facts and some controversy between their supporters. In writing an introduction to the subject it becomes necessary to outline diagrammatically the more important phenomena dealt with and the main interpretations of these in current use; the various points at which the processes of the cotton plant differ from the normal may be noted as briefly.

It is hoped that the following notes will serve to make intelligible the papers referred to, and to translate some of the cytological terms which are in general use in that science, albeit not to be found in the "Concise Oxford Dictionary."

The *Encyclopædia Britannica* defines cytology as the "scientific study of the 'cells,' or living units of protoplasm, of which plants and animals are composed," and gives a lucid outline of the subject in general. A fuller definition is given by Webster as "the branch of biology treating of cells, with reference to their structure, functions, multiplication and life history."

STRUCTURE OF THE CELL

Since the cell is the unit of the plant, it is to be expected that different plants will show differences in their individual cells, apart from mere questions of size and shape, and it is with these differences that modern cytology largely deals. At the same time, so little is known of the processes involved in the life history of the cells, and their methods of division, that it is still of importance to record, and as far as possible interpret, every feature of their life that may be observed.

The cell unit consists primarily of "protoplasm," the basic substance of life in both animals and plants, which is usually encased in a "cell wall" of non-living matter secreted by it. The protoplasm of the cell (or

"protoplast," as it is sometimes called when organised) is a more or less transparent, viscous, granular fluid, of very great complexity from both the chemical and physical points of view, and frequently possessed of the property of movement; it may occupy the whole of the cell, but generally includes several cavities or "vacuoles" containing cell sap.

Part of the protoplast is a rounded body, of denser protoplasm than the remainder, which is known as the "nucleus." The nucleus is the controlling body of the cell, and governs all its activities as far as can be ascertained; when the nucleus divides, the whole cell divides (except in rare cases); and without it the remaining protoplasm (generally referred to as the "cytoplasm") is unable to form a cell wall. Since there is usually little to distinguish the cytoplasm of one plant from that of another, the attentions of cytologists are usually confined to the nucleus.

Methods of Investigation

The method by which these examinations are made is long and involves rather delicate technique. The part of the plant in which the cells are to be examined is placed in a solution which kills the protoplasm and nucleus without altering their minute structure, "fixing" their components in the position where they happened to be at the moment of immersion. The tissue containing the cells is then placed in a succession of fluids leading up to melted paraffin wax, in which the tissue is allowed to set firmly, every part of it thoroughly saturated and imbedded in wax. This wax is then trimmed to form a neat block, which is placed in a "microtome," which cuts it in minute slices as thin as 2.5 micra (μ), or 1/10,000 in., though slices or sections twice that thickness are generally used. The sections, which are usually slightly crumpled by the cutting edge, are allowed to expand on a drop of warm water, and are then fastened to a glass microscope slide with white of egg, after which the slides are immersed in a solvent which dissolves away the wax and leaves the section ready for staining.

Staining is usually done by the delicate hæmatoxylin or logwood technique which bears the name of Heidenhain, and the stained sections are mounted under cover glasses in the usual way, to be examined with all the refinements of modern microscopy. In all, the tissue may undergo up to twenty-five or more operations before the finished sections are ready for the microscope; but the sections are so thin that as many as five may pass through a nucleus one-thousandth of an inch in diameter.

In a section thus prepared nuclei will be found probably in all stages of their development, and it is the task of the cytologist to arrange these stages in their natural order, and to make drawings of the most representative in historical sequence; photography is usually of little help at the very high magnification necessary—up to 3,000 times; the drawings may have an area nine million times greater than the actual cross section of the nucleus drawn.

In nuclei thus stained it is at once evident under the microscope that there are two substances present. The first, which forms the groundwork of the nucleus, is stained very faintly grey, or not at all, and is known as "achromatin," or when it exhibits thread, or mesh-like structure, as "linin." The other substance, which stains dark blue or black with hæmatoxylin, is known as "chromatin," and it is this substance which is responsible for all the characters of the plant, since any one cell is theoretically capable of reproducing the entire plant anew. The changes which this chromatin

undergoes, and the mechanism by which it is equally shared each time the cell divides, are of extraordinary interest. In the nucleus in its normal condition, or resting stage, the "chromatin" exists in the form of a more or less irregular sponge-like network or "reticulum," containing a round, very deeply staining body, the "nucleolus."

CELL DIVISION

Mitosis

In the first phase of cell division, or "mitosis" ("karyokinesis"), which is often referred to as the "pro-phase," the reticulum changes gradually into a definite number of fine threads, which may be separate or may be arranged end to end in a more or less continuous thread or "spireme," which later separates into a definite number of shorter lengths, the "chromosomes." The number of these chromosomes is constant for every cell in every plant of any given species (except the pollen or egg-cells, which will be referred to in detail below), though the chromosome numbers in two species of the same genus may be different.

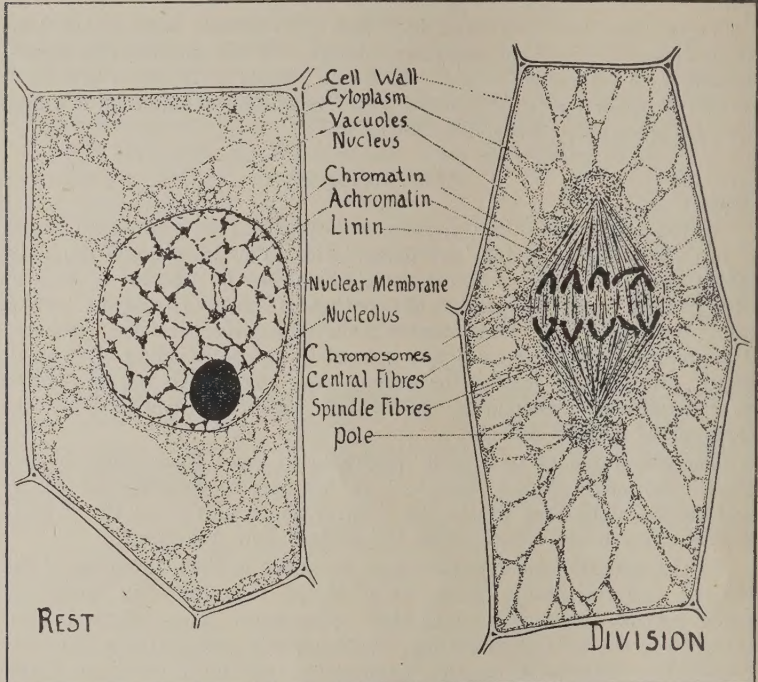
The chromosomes next split longitudinally over their whole length, though the halves do not separate, and then become progressively shorter and thicker until they are in the form of short split rods; the delicate "nuclear membrane" surrounding the nucleus now disappears.

In the meantime changes have been occurring in the achromatin of the nucleus, and two groups of fine linen "fibrils" radiating from "poles" on either side of the nucleus make their appearance. These fibrils become attached to the split chromosomes, which are now arranged in a flat plate ("diaster"). At this stage the chromosomes are most easily countable (since they are all in the same plane), and the whole forms the characteristic "spindle"; the linen portion, which is of some complexity, is known as the "achromatic figure," and the whole stage is known as the "metaphase."

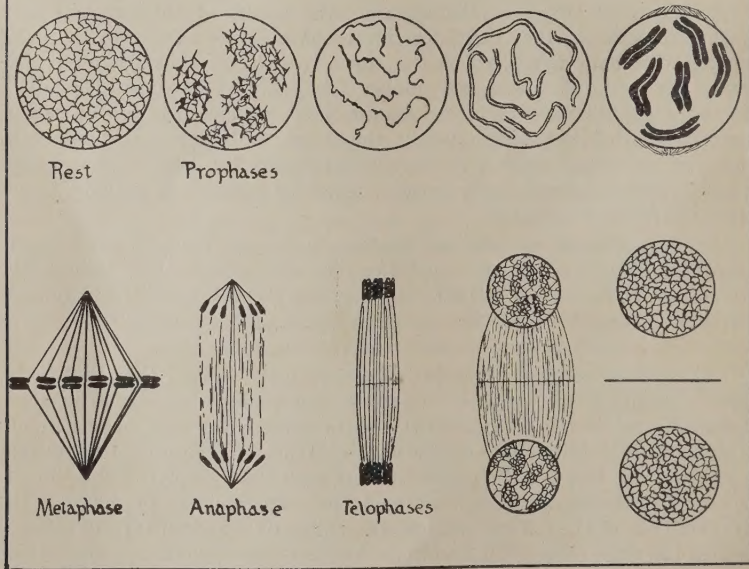
In the next stage, the "anaphase," the halves of the longitudinally split chromosomes, the "daughter chromosomes," move apart to the poles of the achromatic figure, where they form two closely packed groups; between the groups may be seen a number of linen fibres, the "central spindle." In the "telophase" the chromosomes are each reorganised to form a reticulum like that of the original nucleus, the nuclear wall reappears round each, and the nucleolus, which has disappeared during the later stages of prophase, appears anew in each nucleus, where its function is perhaps to act as a reserve of chromatin.

While the nuclei are still in telophase, a new wall is laid down in the central spindle, completely separating the two nuclei and dividing the cytoplasm of the parent cell into two portions ("cytokinesis"); the nuclear division is complete, and the chromatin of the parent cell has been shared with meticulous accuracy between the two daughter cells.

The process of "mitosis" holds good for all the cells of the plant body, the "vegetative" or "somatic" cells. It was found by Beneden in 1883, however, that in the sex cells of the worm *Ascaris*, the number of chromosomes was half that of the somatic cells, and in 1888 Strasburger found that the same was true for the pollen and egg cells ("microspores" and "megaspores") of flowering plants; and that the reduction was produced in the mother cells of the embryo sac and the pollen by a special type of division which has since come to be known as "meiosis," or reduction, to distinguish



MITOSIS or KARYOKINESIS



it from "mitosis" or equal division already described. The two kinds of division are frequently referred to as being respectively "heterotypic" (where the nuclei produced are different in chromosome number from their mother cell), and "homotypic" (where the numbers in mother and daughters are equal).

Meiosis

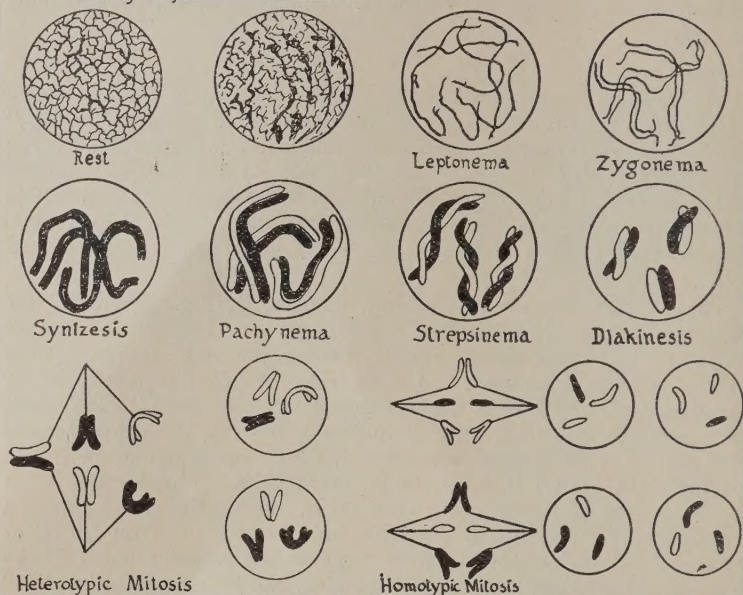
The typical reduction division consists of a heterotypic division closely followed by a homotypic division, the result being four nuclei or "tetrads," each having half the somatic chromosome number. In fertilisation, the egg cell and pollen cell ("megaspore" and "microspore") fuse to form a nucleus having again the full somatic number of chromosomes. The theory of the reduction division is slightly complicated by the fact that there are two distinct interpretations of the process over which some controversy has arisen.

Parasynapsis.—In the first scheme, reduction by "parasynapsis," the nuclear reticulum of the mother cell changes into long slender threads ("leptotene" or "leptonema" stage). During the early prophase these threads unite or "conjugate" in pairs, side by side ("parasynapsis" or "parasyn-desis"), to form a doubled thread, or "zygonema." The whole then contracts to a tight knot ("synizesis"), bringing the paired threads into very close association, and when the knot opens out again ("open spireme") the thread is found to be much thicker ("pachynema"). A certain amount of twisting may then occur, making the paired thread a "strepsinema." The thread shortens and thickens to form "bivalent chromosomes," each consisting of two complete chromosomes united side by side, and of the reduced or "haploid" number, half the somatic or "diploid" number of the mother cell. The bivalent chromosomes are scattered throughout the nucleus in "diakinesis" until the nuclear membrane dissolves and the spindle forms; the two components of each bivalent chromosome are then pulled apart again, and pass up to the poles ("anaphase"), developing a fresh longitudinal split along which they will divide in the ensuing mitosis, which follows immediately, or after a very brief "interphase," and is quite normal.

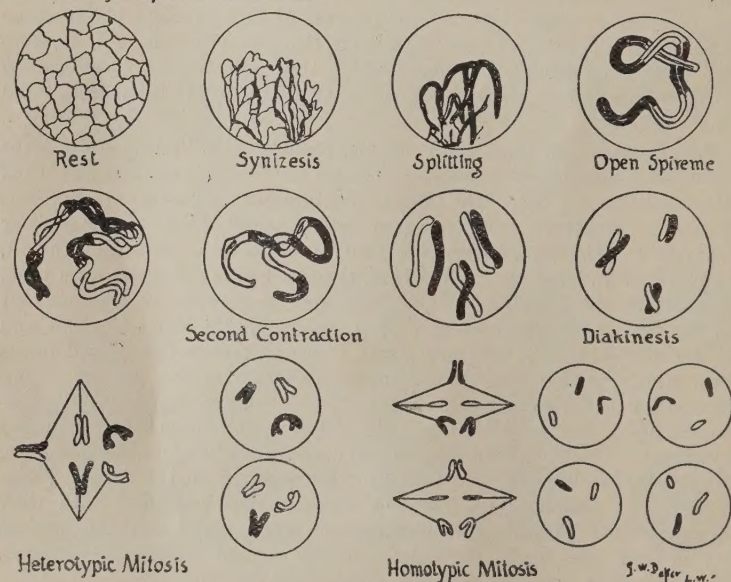
Telosynapsis.—In the second scheme, reduction by "telosynapsis," the distinction is that the chromosomes are held to conjugate in pairs *end to end* rather than side by side. The nuclear reticulum of the mother cell becomes thready in structure and contracts into a tight knot ("synizesis" or "synapsis"). The knot loosens out into the form of a continuous thread, which is double, showing a split which, though it may disappear for a time, reappears in the anaphase. When the knot is completely loosened out and the thread fills the nucleus ("open spireme"), the latter thickens and twists ("strepsinema"), contracts again ("second contraction"), and forms loops, which break apart through a segmentation of the spireme. Conjugation of the chromosomes has occurred "parasynaptically" ("parasyn-detically"), and each loop consists of two split chromosomes arranged end to end. The loops then thicken and condense to bivalent chromosomes, the split being obscured, and take up their position on the spindle ("diakinesis"). In anaphase the bivalent chromosomes pull part into their two constituent (univalent) chromosomes, which may show the original split as they pass up to the poles. In the normal (homotype) division, which follows at once, fission takes place along this line.

REDUCTION DIVISION OR MEIOSIS

A Parasynaptic Scheme



B Telosynaptic Scheme



The figures will perhaps give a clearer idea of the differences of the two schemes than can be conveyed briefly in print, though it must be remembered that they are highly diagrammatic, and that it is much harder to discern the facts in the actual slides. The reduction division is important in cytology not only from the point of settling this very difficult question of the mode of conjugation, but also because it provides an excellent means of discovering the chromosome number of any species; since the numbers are halved at the reduction division, they are easier to count when numerous, and in the case of minute chromosomes, they are seen at their best and largest as "bivalents," particularly in pollen mother cells.

THE CHROMOSOME NUMBER

It is impossible to over-estimate the importance of cytology, and in particular of chromosome numbers, in the breeding of crop plants. The recent work of Karl Sax¹ on wheat hybrids, and Bremer² on sugar cane, is replete with instances where cytology has explained and confirmed the work of the geneticist, and the two papers now presented contain at least one concrete case. For some time past endeavours have been made to hybridise the cottons of the New World with those of the Old, without success. American cottons refuse to cross with Indians, in spite of every refinement of technique. Cytological examination shows at a glance the basis of the difficulty—for whereas the American cottons have 26 chromosomes (haploid number), Indian and Chinese cottons have only 13. This relation of the numbers of different species in a genus is seen to be of common occurrence if reference is made to the exhaustive lists of chromosome numbers published by Ishikawa³ and Tischler⁴, and has been especially studied by Winge⁵.

Other problems which are dealt with by cytology are chiefly concerned with sterility, which is frequent in hybrid plants, and may be found in the inability of a particular strain of plants to produce pollen, or of the pollen produced to fertilise other plants of its own race, though sometimes fertile with plants of another race; such problems are particularly interconnected with the science of genetics. For further details of the scope and meaning of cytology, reference may be made with advantage to the recently published work of Sharp,⁶ from whom the diagrams illustrating this note have been drawn.

CYTOLOGY OF THE COTTON PLANT

The nuclear history of the cotton plant in general follows the lines already described, and the reduction division takes place by "telosynapsis" or "end to end" conjugation, the second contraction being clearly marked. There are several points of unusual interest to the cytologist in the behaviour of the cytoplasm during this division. Shortly after the chromatin arranges itself in the "open spireme" condition, the cytoplasm in a narrow band round the nuclear wall becomes thicker and denser, forming a well-marked "perinuclear zone"; at the same time the remaining cytoplasm takes on a net-like formation, with many of the threads running radially towards the nucleus, after the nature of a spider's web. This perinuclear zone persists throughout the reduction division, when it is replaced by two others which form within it and around the daughter nuclei; it does not occur in the vegetative or somatic cells, but seems to be found solely during the process of pollen formation. The mechanism of diakinesis, the arrangement of the chromosomes upon the spindle, is partly dependent on this zone.

The chromosomes, after they have been segmented off from the thickened spireme, lie partly embedded in the dense substance of the zone, and in good

preparations it can be seen that each chromosome is joined to the others by a network of fine linin threads. These threads appear to contract simultaneously, drawing the chromosomes in towards the centre of the nucleus; and each chromosome as it passes inwards, appears to draw with it another linin strand which is later to become part of the "spindle." At the conclusion of diakinesis the chromosomes form a close circular mass at the centre of the nucleus, with linin fibrils radiating from them in all directions. This condition is called the "multipolar spindle." The spindle ends in the zone now appear to pass up and down towards the poles of the nucleus, and as they move round conditions may occur where they are still found arranged as three or four "poles." Eventually they collect into the two poles of the spindle; the chromosomes form a flat plate between them at the "equator," and anaphase commences.

A third point of interest is found in the manner in which the four grand-daughter nuclei, or "tetrads," are divided from each other without forming a wall upon the central spindle. When the tetrad division is complete, the four nuclei are all embedded in a common mass of cytoplasm, in which the remains of the spindles can still be seen. At this stage small furrows appear at several points on the outside of the cytoplasm between the nuclei, and gradually extend inwards, growing deeper and wider until they form groves which break through the spindle fibres and separate the four nuclei from each other, each nucleus surrounded by cytoplasm. This method of division by furrowing, as distinct from the normal method of division by laying down a wall, is still comparatively uncommon, and has only been described in a few plants, as, for instance, by Farr⁷ in *Magnolia* and *Nicotiana* (the tobacco plant).

The last point is the chromosome number—26 or 13. These numbers are uncommon cytologically, where most plants have chromosome numbers of a multiple of some lower figure. But as Winge has pointed out, chromosome numbers are difficult to count, and there is always an unconscious tendency for the worker to grasp at a "nice" figure, particularly an even and highly divisible number. At the same time 13 and its multiples have been recorded in other species than cotton, and several explanations may be found for their existence from the known facts of nuclear division.

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GLOSSARY

Compare also Figs. 1 and 2.

- Achromatin**—The basic substance of the nucleus which does not stain with Heidenhain's hæmatoxylin solution; includes "linin," *q.v.*
- Achromatic Figures**—The non-staining portion of the spindle, the mechanism of linin threads which is found in the nucleus during the separation of the daughter chromosomes.
- Anaphase**—The stage in nuclear division, both mitosis and meiosis, when the daughter chromosomes separate and pass to the poles of the nucleus.
- Bivalent**—A chromosome (in reduction division) which is formed by the fusion of two dissimilar chromosomes.
- Cell**—The structural unit of plants and animals, theoretically capable of reproducing the entire organism; consists of protoplasm, with or without a limiting cell wall, and various inclusions.
- Cell Wall**—The membrane which surrounds a cell; in plants is usually made of celluloses and pectic substances, and is permeable by most solutions.
- Central Spindle**—The portion of the achromatic figure which still unites the daughter nuclei after the chromosomes have separated in anaphase.
- Chromatin**—A substance found chiefly in the nucleus, which is stained deeply by hæmatoxylin solutions, and which is supposed to carry the hereditary characteristics of the cell and of the organism of which the cell forms part.
- Chromosome**—A small body, rod or bean-shaped, consisting largely of chromatin. The form of the chromosome may undergo various changes during the life of the cell, but the number of chromosomes is constant in any cell of any organism of any given species.
- Conjugation**—The fusion of chromosomes in the reproductive processes.
- Cytokinesis**—The division of the cytoplasm of a cell, as distinct from the division of the nucleus.
- Cytology**—The study of cells, their structure, functions, reproduction and life history.
- Cytoplasm**—The basic protoplasm of the cell, as distinct from the nucleoplasm of the nucleus.
- Daughter Cells**—The product of the first division in meiosis, or reduction. The pollen mother cell gives rise to two daughter cells, which in their turn divide again, forming four "tetrads." The terms "mother" and "daughter" cell are used loosely—describing other cell divisions; the implication of femininity is meaningless.
- Diakinesis**—The stage in the history of the cell division at which the chromosomes move to take up their positions (metaphase) on the spindle, prior to their separation in anaphase.
- Diaster**—The flat plate of chromosomes at the centre of the spindle before anaphase commences. Where the number of chromosomes is united, and their shape allows individuals to be recognised, it is found that this arrangement in diaster is typical in any given species.
- Diploid**—See *haploid*.
- Fibril**—A thin strand or thread of "linin" or "achromatin" which is involved in the mechanism of the nucleus by which the chromosomes are separated.
- Haploid** (single)—The number of chromosomes in the pollen or egg cells is "haploid" half that of the "diploid" number found in the cells of the plant body, i.e., in the "vegetative" or "somatic" cells.
- Heterotypic**—When a cell divides to form two daughter cells containing dissimilar chromosomes, the division is known as heterotypic, or reductional.
- Homotypic**—In contradistinction to heterotypic, when the nuclei arising from a cell division contain similar chromosomes; also known as equational.
- Interphase**—The short pause or resting stage between a heterotypic and a homotypic division.
- Karyokinesis**—The normal method of division of a nucleus, including both "mitosis" and "meiosis," and involving the formation of a nuclear spindle, with consequent equal partition of chromatin in the daughter cells.
- Linin**—That portion of the "achromatin" of the nucleus which is found in the form of threads or fibrils, or even a network.
- Leptonema**—In the earlier stages of the reduction division, the chromosomes are found in the form of long slender threads of chromatin. This is known as the "leptonema" stage, sometimes described as "*leptotene*" by French writers. In the ensuing stage the leptonema threads unite in pairs to form a doubled, thicker thread, the "*zygonema*."
- Megaspore**—The egg cell, or female reproductive cell, which, in fertilisation, fuses with the microspore, the male or pollen cell.

Meiosis—The reduction division of the sex cells as distinct from mitosis, the normal division of the plant body cells. Meiosis occurs only in the sex cells; the new cells produced by this division have only half the chromosome number of their mother cells. Meiosis consists of one "heterotypic" division followed by a "homotypic," *q.v.*

Metaphase—The stage in nuclear division in which the spindle is fully formed and the chromosomes are arranged in "diaster."

Microspore—The pollen cell; see *Megaspore*.

Mitosis—The normal division of the nucleus, in which two new nuclei are produced having the same chromosome number and complement as their parent and each other.

Multipolar Spindle—A stage in diakinesis in which the spindle ends have not yet taken up their final position at the poles of the nucleus, and the appearance of a tripolar or quadripolar spindle is found.

Nucleus—A portion of the protoplasm of a cell, differentiated from the remaining cytoplasm by its density, its *nuclear wall* and its staining properties. The nucleus is understood to be the controlling organ of the cell, and to carry its hereditary characters, and in every case initiates cell division.

Nucleolus—A densely staining spherical body, which is found within the nucleus in its resting stage, and disappears in prophase. It is understood to be a reserve of chromatin, but its exact function is still obscure.

Protoplasm—The basic substance of all life on this earth. A slimy, viscous, colloidal mixture containing up to 95% water, and largely proteid in its reactions. Its composition is extremely complex, and has not yet been solved.

Pachynema—A stage in the reduction division in which the *zygonema* shortens and thickens to form a thick thread, which may show further twisting (*strepsinema*).

Parasynapsis—Side by side conjugation of the chromosomes, in early reduction division, as distinct from *end to end* conjugation (*Telosynapsis*). Considerable controversy has taken place as to which actually occurs.

Perinuclear Zone—A layer of dense cytoplasm which surrounds the dividing nucleus in a number of plants, and appears to contribute to the formation of the spindle.

Prophase—The early stages of nuclear division, from the resting stage to the segmentation of the chromosomes.

Reticulum—A network or sponge-like structure, which may occur when two dissimilar substances are so mixed that each interpenetrates the other.

Somatic—Equivalent to vegetative; containing the diploid number of chromosomes; relating to the plant body.

Spindle—The typical figure formed by the linen fibrils and the chromosomes in metaphase and anaphase.

Spireme—The continuous thread formed by the chromosomes in the prophases of a nuclear division. According to the looseness or tightness with which the thread is found in the nucleus, the stage is known as "open" or "close" spireme.

Synapsis—In early prophase the thin chromatin threads (*leptonema*) contract into a tight knot, known as the synaptic knot. A number of changes are suspected to take place during this synapsis, but there is little clear evidence as to what actually occurs before it loosens out again as a continuous thread or spireme.

Synizesis—Another term for Synapsis.

Strepsinema—A twisted condition of the spireme prior to its segmentation into chromosomes.

Telophase—The last stage in nuclear division; the chromosomes pass up to the poles and recombine to form a reticulum; the nuclear wall reappears round the new nuclei; and a new wall in some cases is begun between them.

Telosynapsis—End to end conjugation of the chromosomes in early reduction division, as opposed to side by side conjugation (*parasynapsis*).

Tetrads—The four pollen cells resulting from the heterotypic and homotypic divisions of meiosis. Also used for bivalent chromosomes in particular cases where the lines of the second split are prematurely visible.

Vacuole—A cavity in the cell wholly enclosed by cytoplasm and usually containing cell sap.

Vegetative—See *somatic*.

Zygonema—See under *leptonema*.

XX.—THE CYTOLOGY OF THE COTTON PLANT

i.—MICROSPORE FORMATION IN SEA ISLAND COTTON

By HUMPHREY JOHN DENHAM, M.A.(OXON), F.R.M.S.

. (The British Cotton Industry Research Association.)

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INTRODUCTION

In the present paper the cytology of pollen formation in Sea Island cotton (*Gossypium barbadense* var. *maritima*, Watt¹) is briefly described, with special reference to the reduction division of the pollen mother-cell and chromosome number. These observations form part of a series of investigations on the cytology of the various cottons in cultivation; their bearing on the problems of scientific cross-breeding and improvement of commercial cottons forms the subject of a separate paper in preparation and therefore need not be discussed here.

Two papers only have been published on the cytology of *Gossypium*, neither of which deals with the type under consideration, though the forms discussed are sufficiently homologous to admit them for comparison. The first, published by Cannon² in 1903, deals with the cytology of a hybrid between commercial Sea Island and Upland plants, with special reference to the production of nuclear abnormalities by special crossing. The cytology of the parents was apparently not studied, but the general outline of the nuclear history agrees fairly consistently with the known behaviour of the sporocyte in other Angiosperms, and with the observations below. Some 19 figures are given, many of them of abnormal conditions, and the chromosome number of the hybrid is stated as 28 (haploid).

The second paper, by Balls³ in 1910, which deals with the cytology of the Egyptian *Mil Afifi* cotton (a commercial type approximating to *G. barbadense*), is chiefly an attempt to establish a mechanism of nuclear division which would account for the absence of the centrosome in the higher plants, and describes certain "thread-ring" structures which are supposed to unite the ends of the spindle fibres, persisting through the life of the nucleus, and taking an important part in the behaviour of the spireme; one plate is given and the haploid chromosome number is stated as 20. This "thread-ring" theory of karyokinesis seems to have escaped the notice of later writers, and the structures have not been described in any other plants. Details of the developmental history of the flower and of the micro- and mega-sporangia may be found in an earlier paper by the same writer.⁴

MATERIAL AND METHODS

Some 200 plants were available in the Shirley Institute experimental greenhouse, raised from seeds of pedigreed pure strains which had been brought from St. Vincent by Dr. S. C. Harland. This material had in every case been self-fertilised for at least six generations, and was in consequence homozygous to a very high degree. A certain amount of material from other sources was also available for comparison, including Trinidad native types, commercial Uplands, and Egyptian *Mit Afifi*, though no haploid material of the latter was obtainable during the period of these investigations. A large number of fixing solutions were tested on buds and root-tips, and it was found that the most satisfactory preparations were given by von Tellyesniczky's potassium dichromate-acetic acid mixture; the addition of small quantities of osmium tetroxide gave slightly sharper chromosome definition, but could not be used in all cases on account of the presence of fats and lipoids. Fixation was carried out at the temperature of the greenhouse, the buds being first stripped of their involucres to facilitate rapid penetration of the fluid. No improvement was produced by fixing under reduced pressure. For dehydration, the recently published technique of Mlle. Larbaud⁵ was used, in which commercial butyl alcohol is substituted for the higher grade alcohols and xylene mixtures, with very marked improvement. Approximately correct diameters of buds for fixing are as follows (involucres removed)—

Synapsis, 3 mm.; diakinesis, 3.5 mm.; tetrad spindles, 4 mm.

When a considerable number of buds had been cut, it was found that late anaphase stages were almost completely absent. It appears from the researches of Laughlin⁶, that in *Allium* root-tips the anaphases are accomplished considerably more quickly than the other stages, and it would appear that in the present case chromosomes entering on anaphase pass into telophase before they are reached by the fixative. To overcome this, a modification of Carnoy's fluid was used (ethyl alcohol 40%, glacial acetic acid 40%, chloroform 20%) with complete success. The material after fixation was taken through a graduated series of alcohol-chloroform mixtures into pure chloroform, to which paraffin wax was added gradually, and finally transferred to pure paraffin wax for embedding. This gives excellent nuclear fixation, though slight distortion of the cytoplasm may sometimes occur. In a few cases, Bouin's fluid was used on account of the sharp chromosome plates which it is said to give, though no improvement on the dichromate fixative could be observed.

Sections were cut at thicknesses ranging from 2–8 μ on a Leitz–Minot microtome. The most satisfactory stain for general purposes was the standard Heidenhain iron-haematoxylin, though Bolles Lee's⁷ recent technique for rapid staining and differentiation was used in a few instances, and Ehrlich's haematoxylin was employed for temporary preparations. Gentian-violet (Gram) gave a few very satisfactory preparations of multi-polar stages. Sections were in the main mounted in euparal, which seems to give sharper definition than Canada balsam, with slight loss of depth of focus due to its lower refractive index (1.483).

On the whole, the material was rather capricious as regards fixation, and a large number of buds had to be cut to secure a representative series of nuclear stages, though little difficulty was experienced in obtaining crisply stained and well differentiated preparations in properly fixed material.

Several different stages can usually be found in different anthers of the same bud; such variation is greater in the winter months than in the summer, and November buds show all stages from synapsis to tetrad formation.

CYTOLOGICAL HISTORY

The Archesporium

In *Gossypium*, as in other species of the *Malvaceae*, the bilocular reniform anthers are arranged in typical looped "festoons" on a staminal column, the lowest five being theoretically sterilised to form petals. In the earliest stage of the development of this column the festoon arrangement can be clearly distinguished, but as the anthers are carried up by the staminal column they can still be recognised as forming five double vertical bands of primordia; with further extension the arrangement becomes obscured, the anthers forming a densely-packed layer on the surface of the column, though in cross-section the five double rows can still be seen. Owing to this packing of the anthers their natural orientation is obliterated, and in cross-sections the loculi will be cut at every possible angle.

The first differentiation of the anther primordia occurs when the bracteoles are sufficiently developed to meet at the bud apex and form a protective sheath. A true epidermis is first formed, increasing by lateral division and persisting in the mature anther. The archesporium proper arises from the sub-epidermal layer, localised areas of which divide to form three well-marked zones of cells, the innermost giving rise in due course to the pollen mother-cells. The morphological value of the archesporium is low and it is almost impossible to distinguish the particular sub-epidermal cells from which these layers are formed, or to follow the process of nuclear division at this stage, on account of the small size of the nuclei and the large somatic number of the chromosomes, but numerous well-defined spindles and plates can be seen, showing the planes in which cell-division is occurring.

The Premeiotic Division

The cells in the third layer differentiated by the archesporial divisions enlarge rapidly, while the surrounding layers continue to divide, and now appear in longitudinal section of the loculus as a definite band of tissue; the cytoplasm is dense and granular, and there are no vacuoles. When they have attained a diameter of about $14\ \mu$ they put in one final division, the premeiotic, from which they emerge as pollen mother-cells.

Owing to the rapidity and the fugitive character of this division, together with the large number of chromosomes and their small size, it is difficult to determine the exact sequence of events at this stage. The most striking feature is the definite increase in the amount of chromatin which takes place in the prophase; this, as in the case of *Primula* investigated by Miss Digby⁸, appears to be produced by a process of budding from the nucleolus, which shows a very marked reticular-alveolar structure. During the earlier part of this phase the nucleolus often appears to be held in place by one or two bands of achromatic substance, which cross the nucleus diametrically (Plate I, Fig. 1); when a complete nucleus is found in a thick section, a characteristic "cross" is seen. As the nucleus enlarges, more and more chromatin beads are seen, strung out on a fine achromatic reticulum which simultaneously thickens and becomes more visible (Fig. 2). The chromatin joins up into a continuous looped spireme, which fragments in diakinesis, the segments (in which no split can be discerned) becoming shorter and

thicker (Fig. 3). There is no evidence of triple spindle formation such as occurs later, in the meiotic division. The nucleolus in prophase keeps its size, but becomes progressively less chromatic and finally disappears at the diakinesis (Fig. 4).

In the metaphase (Fig. 5) the chromosomes form a thick plate, and in diaster the individual diads can be seen as short rod-shaped bodies, of the same shape as those of the root-tip mitoses, and markedly different from the coccoidal form which they assume in the meiotic and post-meiotic divisions. Spindle-formation occurs with faintly visible polar differentiation. There are no signs of the peculiar thread-ring structures described by Balls³ in both somatic and haploid cells.

In the anaphase (Fig. 6), the polar bodies become temporarily more pronounced; the chromosomes, as they pass up the spindle, may show the usual V- and Y-forms, though these cannot readily be resolved. There are usually several laggard chromosomes, and in these cases it may occur that two or more are seen joined end to end and passing up the same fibril; this would seem to point to an incomplete separation of individuals in diakinesis.

In telophase (Fig. 7), a slight condensation of the chromosomes occurs before the first sign of the inception of the partition wall in the phragmoplast, but with the formation of the nuclear wall the chromosomes separate from one another (Fig. 8) and, as far as can be distinguished, join up to form a continuous spireme by a fresh fusion of the chromatic substance. As the spireme loosens out, the chromatin segregates into small beads along it, while at the same time one or two small karyosomes appear (Figs. 9, 10).

The nucleus then passes into the resting stage, and the beaded spireme moves outward to the nuclear periphery, while the chromatin is apparently resorbed by the nucleolus, which increases rapidly in size; if two nucleoli are present, fusion apparently takes place, since only one persists. The structure of the nucleolus is obscured by its greatly increased affinity for stains, but there are distinct signs of alveolation (Fig. 11). A few small beads of chromatin remain in the achromatic reticulum during the resting stage.

Meiotic Prophase

The first indication of the onset of the reduction division is given by the separation of the cytoplasm of the pollen mother-cells from the surrounding tissue (though in most cases the cytoplasm continues to adhere to the mother-cell partition walls), so that they swim more or less freely in the locular cavity (Plate VII., Fig. 1). This appears to be due to a definite contraction of the cytoplasm; it was observed with all the fixatives used, and has been recorded by both Balls and Cannon. No reference to a similar contraction in other material is forthcoming, except in the case of *Syringa*, which Juel⁹ considered an abnormality:

Simultaneously with this contraction, the tapetal layer begins to divide; in many cases no wall is laid down between the new nuclei, with the result that many tapetal cells are binucleate. As in other plants, the nuclear divisions are very irregular, and many abnormalities can always be found in the tapetum of otherwise normal anthers.

The first sign of renewed nuclear activity is found in the thickening and proliferation of the achromatic reticulum, while the sparse chromatin beads already present enlarge and become more prominent (Plate I., Fig. 12). The

achromatic reticulum develops rapidly as the cell enlarges, filling the nuclear cavity with its ramifications (Figs. 13, 14), while the chromatin beads break up into smaller granules which pass out into the new network, where they mark the intersection of the strands. At this stage signs of pairing become evident, though it is not clear how far this parallelism really exists and how much is due to the superposition of strands at different depths in the nucleus.

Synapsis

The synaptic contraction comes on rather suddenly and it is difficult to find a full series of intermediate stages between the fully developed reticulum and the synaptic knot. The first indication of approaching synizesis is given by the loosening away of one leptoneima from the nuclear wall on the opposite side to the point of contraction, with a slight massing of the reticulum about the latter (Plate II., Fig. 15). In complete synapsis the knot is apparently bounded by the strands which had previously been peripheral. Slight changes can be made out in this bounding layer, in thin sections, during synapsis, but it is impossible to resolve the centre of the knot; no loose ends or loops can be seen in knots which have not been touched by the knife. The nucleolus lies to one side of the knot and partially embraced by it (Plate II., Fig. 16, and Plate VII., Figs. 1 and 2).

In the loosening-out stage the knot becomes less granular and more fibrillar in appearance. Loops appear at the edges, in which chromomeres and a longitudinal split can be made out, pointing to the fact that syndesis has occurred and that the spireme is now zygotene. The nucleolus is pushed out of the loosening knot and lies caught up in the looped spireme (Plate II., Figs. 18, 19); its structure is now much more homogeneous and in most cases no alveolation can be observed when the loosening is complete, nor has any chromatin budding been observed from this stage onwards. The spaces in the synaptic knot are still occupied by an achromatic substance which disappears as the loops emerge (Plate VII., Figs. 3, 4, 5).

In view of the importance of the changes which take place during synapsis, every effort has been made, by varying staining and fixation, to obtain preparations in which details of the changes in the synaptic knot might be observed. Synapsis is here a phase of relatively long duration, since it is possible to cut buds in which every nucleus shows close synizesis, whereas in other buds, both younger and older, several different stages may be observed simultaneously. It has not been possible to follow more than the changes which take place at the extreme edge of the knot, however, even in the thinnest sections, and at the extreme limit of microscopic resolution.

The following tentative observations were made—

(1) At the completion of the contraction the knot is bounded by the leptoneima which was originally peripheral to the nucleus.

(2) The achromatic substance condenses to form a sponge-like reticulum, the spaces of which are occupied by a hyaline plasma; the chromatin is precipitated in this plasma in the form of fine granules, which are presumably free to move in the interspaces of the achromatic reticulum.

(3) The synaptic knot expands slightly and the achromatin takes on the form of a single tangled thread, with no sign of a split.

(4) The chromatin granules are redeposited from the hyaline plasma on to the thread in a fairly even layer. They then appear to pass along the thread, forming rounded aggregations at irregular intervals.

(5) The thread splits between these aggregations of chromatin and as the split extends they pull apart into apparently equal portions or chromomeres.

The theoretical aspects of synapsis have been considered in some detail by holders of the parasynaptic theory of conjugation; under their scheme, the association of parallel threads before and during synapsis represents the paring of entire chromosomes to form *bivalents*, which after much shortening and thickening are separated in the heterotypic mitosis, during the anaphase of which (or earlier in the case of chromosome tetrads) the split that is to function in the homoeotypic mitosis makes its appearance (Sharp¹⁰). Under the telosynaptic theory of conjugation the splitting of the thread represents the division of *univalent* chromosomes along the line which will again become evident in the heterotypic anaphase and the homoeotypic mitosis; this splitting may have had its origin in the premeiotic telophase. The *bivalent* chromosome is in this case formed by the association in pairs (at first end to end in the spireme, but later side by side) of segments of this split spireme at the time of the second contraction.

Conjugation in *Gossypium* is clearly of the telosynaptic type, as can be seen from the later behaviour of the spireme, and this observation seems fully confirmed by Cannon's account² of the formation of the chromosomes, published, however, before Farmer and Moore¹¹ had given the first definitive account of the process, or Farmer¹² had emphasised the essential differences between the telosynaptic and parasynaptic theories. The theory of the telophasic splitting, found by Miss Digby in *Galtonia*¹³, *Primula*⁸, *Crepis*¹⁴, and especially *Osmunda*¹⁵, and by Miss Fraser in *Vicia*¹⁶, here lacks direct confirmation, though such splitting is thought to occur in the meiotic telophases.

In Miss Digby's description of the synapsis of *Osmunda*¹⁵, in which it can be observed with exceptional clearness, it appears that the thread structure of the half univalent chromosomes is never obscured while the association of the threads and their sorting out is effected, nor is there any change in the distribution of the chromatin along them. Considerable divergence of opinion exists as to the nature of the chromomeres. Strasburger^{17, 18} held that these are complexes of the "pangens" of de Vries, interchanged during conjugation. Allen¹⁹, in an account of the parasynapsis of *Lilium*, holds that the fusion of the leptotene threads involved the fusion of the chromomeres, which he took to be composed of still smaller chromatic elements, the resplitting of the pachytene thread being started by the redivision of the chromomeres. Gregoire²⁰, on the other hand, denies the individuality of the chromomeres, which he considers merely as thickened portions of an unevenly stretched thread; Wenrich²¹, however, has found in the grasshopper *Phrynotettix* that in any given chromosome the chromomeres show a remarkable constancy in size and arrangement in different cells and even different individuals. In the present instance the precipitation and redispersion of the chromatin granules on the univalent thread would seem to agree with the observations of Allen, with the exception that the split is initiated not in the chromomeres but in the thread or univalent spireme. It is obviously impossible, in view of the length of the spireme and the large number of chromosomes, to identify the arrangement of the chromomeres. In the majority of preparations the doubleness of the thread cannot be made out except in short lengths, which can be found in all stages

up to the second contraction, and even later. There is no evidence that any true fusion of the threads takes place, though the closely associated halves may appear at first sight as a single thick thread; at the same time there is nothing to prevent the formation of the "chiasmatypes" of Janssens,²² with their important bearing on genetics. The loosening out of the univalent spireme from the synaptic knot is very much more gradual than the previous contraction, and agrees very closely with the process as reported in *Primula floribunda* by Miss Digby.⁸ The loops emerging from the knot are for the most part obviously univalent, though occasional portions are twice as thick as the remainder, showing marked twisting, with frequent longitudinal fission. This points to a certain amount of chromosome association already having taken place in the synaptic knot, with precocious formation of bivalents, though it is usual for this to occur only in the later second contraction.

As the nucleus passes into the well-known "open spireme" stage (Plate II., Figs. 20, 21) the looping becomes more and more accentuated until the knot is entirely unravelled and the loops have reached the periphery. At points of contact where there is anastomosis of the univalent strands there is always a chromatin bead or swelling (Figs. 22, 23). In some nuclei there is evidence of considerable tension on the strands which cross the centre; they are drawn out quite straight and all signs of splitting are obliterated (Plate VII., Figs. 8, 9).

Examination of serial sections of open spireme stages shows that the spireme is quite continuous; the loops pass freely through and round each other, and there is very little anastomosis. In this condition there are no signs of any parallel arrangement of the strands, except for occasional bivalent lengths of thread. The height of this condition is reached when the whole of the spireme has passed out to the periphery and there are no longer any strands crossing the centre of the nucleus (Plate III., Fig. 24).

With the formation of the hollow spireme the structure of the cytoplasm begins to show marked changes. The nucleus moves from the side of the cell toward the centre, and the dense and rather granular cytoplasm grows progressively more reticulate in a radial pattern, increasing in density centripetally (Plate VII., Figs. 11, 13).

The hollow spireme must not be regarded as a condition of nuclear equilibrium; it appears that there are continual changes taking place in the arrangement of the loops, marked by an increase of the amount of anastomosis, and a general thickening of the whole thread, leading up to the second contraction of the spireme, in which the univalent chromosomes, united at their ends, are brought into the bivalent condition by the formation of loops.

Second Contraction

The onset of the second contraction is extremely variable and in a few cases it has been observed that it is omitted entirely, the nucleus passing direct from the open spireme to the segmentation of loops. In general, however, the loops of the open spireme withdraw from the periphery of the nucleus to one side (Plate VII., Figs. 2, 5 and 10); the spireme grows thicker and shorter, staining more deeply, and the chromomeres and chromatin beads disappear. At the height of the contraction the spireme can be seen as a tangled thread at one side of the nucleus, very much twisted and looped,

but with none of the obliteration of structure which is characteristic of synapsis (Plate III., Fig. 26). The nucleolus (which already is becoming markedly less chromatic in its staining reaction) lies outside the contracted thread, but connected with it by two or more strands, and the whole occupies rather more than half the nuclear cavity. At the same time the cytoplasm shows an increase of the radial reticulation already referred to, and the formation of the typical perinuclear zone is begun.

As the spireme comes out of the second contraction it is evident that a very decided contraction and twisting has occurred and is still continuing, with the formation of a "strepsinema" (Figs. 27, 28). Careful examination of these twisted portions reveals the presence of very fine achromatic strands uniting adjacent threads in a ladder-like form, and as soon as the loops of the twisted thread reach the nuclear wall segmentation of the twisted loops commences. The segmentation and condensation of the spireme into chromosomes has been described by Cannon,² though his account has been questioned by Balls³; from the description and the figures presented it can be seen that the process agrees almost exactly with the present observations, with the sole exception that no ring-shaped chromosomes have been seen in metaphase, nor do they appear to have U-, V-, or X-shapes in anaphase.

As the loops shorten and contract they break away from each other and assume the form of irregular rings (Figs. 29, 30, 31). Conjugation has occurred telosynaptically, and each ring consists of two split (univalent) chromosomes arranged end to end; the split in the univalent halves may often be visible right up to the final condensation of the ring into the bivalent chromosome. Cannon's observation that "in any nucleus the loops and rings are of a uniform size" has not been confirmed, except in so far that no marked separation into two groups of different sizes can be made out. As the central split is obscured by further contraction and diakinesis commences, the bivalents become more uniform in shape and size.

Diakinesis

The phenomena of diakinesis and spindle formation are intimately connected with the perinuclear zone already referred to. This zone appears to be typical for microspore division in *Gossypium*, and is possibly characteristic of the Malvaceae in general; it is found in both the meiotic heterotype and homotype mitoses, but not in the premeiotic or somatic divisions. The formation of such a perinuclear zone and its bearing on the development of the spindle structures has been studied in great detail by Miss Byxbee²³ in *Lavatera*, and has been observed also by the writer in *Althaea*. Cannon makes reference to records of such zones in *Lilium* by Mottier,²⁴ and in *Larix* by Belajeff,²⁵ but these on examination seem scarcely comparable. More allied conditions have, however, been recorded by Jucl²⁶ in *Hemerocallis*, by Hus²⁷ in *Cassia*, and by Mottier²⁸ in *Staphylea*, while similar structures have been figured in a number of other plants, and reference should be made to the recent work of Taylor²⁹ on the genus *Acer*.

Up to the point of diakinesis the process in *Gossypium* agrees closely with that described in *Lavatera*. The cytoplasm in early prophase is of a normal granulo-reticular structure, slightly looser at its periphery than round the nucleus, but fairly homogeneous in structure throughout the cell. Shortly before the nucleus passes into open spireme the cytoplasm appears

to segregate into two constituents, the one granular and the other fibrous, the former having a faintly greater reaction to cytoplasmatic stains, and while the fibrous component loosens out into an irregular meshwork radiating outwards from the nucleus as centre, the granular constituent passes inward toward the nuclear wall, to form a dense layer, the structure of which cannot be resolved accurately, but which appears to be very densely reticular. In *Lavatera* this zone shades off gradually into the radial-reticulate meshwork, but in *Gossypium* the transition is more abrupt; in either case a certain breakdown of the regularity of the radial reticulum occurs when the granular zone is completely formed (Plate VIII., Fig. 14).

From Miss Byxbee's account it appears that spindle formation takes place in the following manner. The meshes of the network, close to the nuclear wall, pull out in a direction parallel to the wall, forming a felt of fibres about the nucleus, while the granular constituent of the cytoplasm collects in a wide dense zone about the latter. This perinuclear zone remains quite distinct from the nuclear wall, and a mass of linen fibres forms in the intervening space, penetrating into the nuclear cavity on the breakdown of the nuclear wall at one or more points. These fibres, and others formed within the nucleus, together form a complex in which the chromosomes are distributed, and from it a multipolar spindle is projected; two of the cones of this spindle become more prominent than the others, which they finally absorb, thereby forming the bipolar spindle. The spindle has sharply-pointed ends, which appear to be inserted in the perinuclear zone, and in early telophase there are traces of granular radiations from the poles, faintly suggestive (in the figures) of centrosomes. In the homotype division the process is repeated more or less exactly.

The chief point of difference between the spindle formation in *Lavatera* and in *Gossypium* is that in the latter there is no separation between the nuclear wall and the perinuclear zone. As the chromosome loops condense and pass out to the nuclear periphery, the nucleolus disappears and, shortly after, the nuclear wall is absorbed. The chromosome rings can at this stage be seen partly embedded in the perinuclear zone, which begins to take on a distinctly fibrillar structure in the neighbourhood of each chromosome mass, the fibrillae radiating outwards through the zone. At the same time, other fibrillae can be seen uniting the various masses (Plate III., Figs. 32, 33). Here, as in *Lavatera*, it is apparent that the fibrillae are from two different sources, the radiating fibrils originating cytoplasmatically from the perinuclear zone, and the connecting fibrils probably from the nuclear linen, increased by the absorption of the nucleolus, which has been held by several observers to act as a reserve of fibrillar material (Strasburger^{30, 31} and Eisen³²). A similar stage has been described by Taylor²⁹ in the microsporocyte of *Acer Negundo*.

It is believed that the migration of the chromosome masses from the periphery to the centre of the nucleus is brought about by the contraction of the connecting fibrils with the simultaneous elongation of the radiating or spindle fibrils (Plate VIII., Fig. 15). (Traces of the connecting fibrils can still be seen in diaster as a fine reticulum uniting the chromosomes.) The whole body of the chromosomes in diakinesis passes evenly and simultaneously to the centre of the nucleus, where at first they form a dense and almost spherical aggregation (Plate IV., Fig. 34; Plate VIII., Figs. 16, 17), though an occasional laggard chromosome may follow at some distance, and the

fibres of the multipolar spindle are evenly distributed over the whole nucleus as radii from this centre.

In the transition from the multipolar to the bipolar condition (Plate IV., Figs. 35-38) there does not appear to be any absorption of these fibres as described in *Lavatera*, but rather a movement of the peripheral ends of the spindle fibres in the perinuclear zone towards two opposite poles, and as the fibres pass round towards these, quadripolar and tripolar stages may be found. Simultaneously, the chromosomes are pulled round from the spherical aggregation into the typical flat plate or diaster of the metaphase. Traces of the connecting fibrils can still be seen in diaster as a fine reticulum uniting the chromosomes (Plate IV., Figs. 42, 43, and Plate VIII., Fig. 21).

The Meiōtic Division

It is difficult to distinguish with certainty the point of insertion of the spindle ends in the perinuclear zone and their attachment; the spindle is usually sharp-pointed and in no case has there been any evidence of the presence of a thread-ring uniting the ends of the fibrils as described by Balls. In good preparations there are definite indications of a very finely reticulate substance in the space between the spindle and the perinuclear zone, particularly in late anaphase, which possibly corresponds to the karyolymph described by Devise³³ in *Larix* and Miss Nothnagel³⁴ in *Allium*. The spindle ends frequently at points which are not diametrically opposite on the perinuclear zone, and in such a case a curved spindle results.

The chromosomes in typical diaster are coccoid in form, rarely showing a faint line of fission, and their number can be counted as 26, though two chromosomes appear to be markedly larger than the remainder; this question will be discussed in greater detail below.

The first appearance of the commencement of the anaphase is indicated by a ring-shaped constriction round each chromosome, and as the two halves are pulled apart the ring forms figured by Cannon may sometimes be seen. The daughter chromosomes in early anaphase are extremely irregular and even angular in shape, and in many cases three and even four fibrils can be seen attached to each, the chromatin being drawn out to a slight point at their insertions. As the chromosomes are further drawn out there may be a further separation of the halves of the univalents indicating the division which will take place in the succeeding homotype mitosis. This separation is, however, extremely irregular, and occasionally the bivalent chromosome is pulled out into a line of about six irregular masses of chromatin (Plate V., Fig. 44). As the univalent chromosomes pass up the spindle the irregularities of shape disappear and the coccoid form is reassumed; in many nuclei (as in the premeiotic division and in diakinesis) there are one or more chromosomes which lag behind the others (Plate IV., Figs. 39, 40), the bivalents in such a case sometimes remaining undivided when the other univalents are in late anaphase.

Some difficulty has been experienced in tracing the transition from anaphase to telophase, owing to the rapidity with which the former takes place. The first sign of the telophase commencing is seen in a slight decrease of the density of the perinuclear zone, together with a thickening of the spindle fibres, which bulge out between the daughter nuclei into a barrel-like figure. At the same time there is a distinct change in the spindle ends, which, from a definite pointed aggregation passing into the perinuclear

zone, retract centripetally, while radial fibres can be seen spreading from them inside the zone, though they have not been seen to pass into the cytoplasm as described by Cannon. The cytoplasm surrounding the nucleus loses its radial arrangement and becomes loose and ragged, contracting slightly, and the collapse of the outlying reticulum may give rise to thread-like thickenings parallel to its periphery.

The chromosomes meanwhile break up into granules; it is impossible to count their number, or to identify them in any way as prochromosomes, and the granules are next seen arranged in a light reticulum (Plate V., Fig. 46), apparently passing directly into a thin continuous spireme which is double (Fig. 50), while a limiting nuclear membrane forms. In some of the preparations examined, a very short resting-stage was apparently interpolated, in which the chromatin seemed to flow along the linin reticulum, giving the appearance shown in Fig. 47. This stage, though figured by Cannon, is probably an abnormal condition; in most cases the chromatin aggregates again into beads of irregular sizes, arranged on linin strands which appear to be continuations of the fibres of the original spindle (Figs. 48, 49).

The reconstructed nuclei lie a short distance inside the points of the original spindle, and there are no indications of a cell-plate formation in the latter. The original perinuclear zone expands and gradually disappears, and simultaneously similar zones are formed within it round each of the daughter nuclei, commencing as soon as the nuclear wall is laid down. The formation of these zones is on the lines already described, and the cytoplasm in their neighbourhood again takes on the typical radial structure; there is evidence for believing that part of the zone arises direct from the original spindle fibres (Fig. 51).

The spindle and the remains of the first perinuclear zone persist for some time and may still be seen when the tetrad cells are rounding off.

A point of some interest at this stage is found in the degradation of the cell-walls separating the original pollen mother-cells, anticipating the breakdown of the tapetal layer at a later stage. These walls may be seen in all degrees of degeneration, with a typical granular appearance, though in no instance do they disappear entirely, traces still remaining when the pollen grains are completely formed; simultaneously a clear hyaline layer is deposited round the periphery of the cytoplasm (Plate VIII., Fig. 23). The nature of this layer is obscure and it was thought at first that it might consist of the "callose" described by Mangin³⁵ in similar conditions. It is not soluble, however, in sodium hydroxide, nor does it stain with Mangin's corallin solution; it stains deeply with Orange G, and gives a deep violet colour with Delafield's haematoxylin. It is more probable, from the role that this hyaline layer plays later in the separation of the pollen cells and the formation of their walls, that it is a complex of a mucilaginous consistency formed partly by the degradation products of the parietal walls and partly by a secretion of the cytoplasm itself. There is no evidence of any secretion from the tapetum at this stage other than the normal tapetal fluid in which the pollen cells are presumably floating.

Homotype Division

In material which shows resting stages between the heterotypic telophase and the homotypic prophase, the chromatin passes from the reticulated or

the beaded state into the double spireme already mentioned, and segmentation takes place without any looping of the thickened thread, the dyads forming by simple condensation and passing direct to the centre of the nucleus, where they form the usual plate. The process by which this is effected has not been fully observed, but there are signs of a multipolar spindle of the type already described. In some of the preparations made when the plants were in very active growth it appeared as if the chromosomes were passing direct from early telophase, without any change of form, to the homotype metaphase, no intermediate stages being found, though this may have been due to the rapidity with which such changes were proceeding.

As the chromosomes split and pass up to the poles, their outline is much more circular than in the heterotype division, and they do not appear to have the short rod form described by Cannon. No further split is visible at any stage of the anaphase, nor have any cases of the stringing out of the chromatin on the fibril been seen as in the meiotic anaphase. The telophase proceeds as before; the chromosomes disintegrate into granules, which recombine into irregular beads in the resting nucleus, and one or two small nucleoli are formed. At the same time, a new perinuclear zone is laid down which persists during the formation of the wall of the pollen grain. This zone is very much more granular than the previous rings, and expands gradually until in the mature grain it lies at the boundary of the cytoplasm; the separation of the zone from the nuclear membrane occurs shortly after the separation of the pollen cells from each other and before the wall is begun.

The pollen tetrads are rather irregularly arranged, generally in tetrahedra, though occasionally all four nuclei are found in a flat plate. Cytokinesis is brought about by furrowing, somewhat on the lines described by Farr³⁶ in *Magnolia*, although there are not always traces of the commencement of the constricting furrow after the first mitosis,* and the existence of the six spindles connecting the four nuclei each to each, a conspicuous feature of the process in *Nicotiana*³⁷, is only faintly discernible. Small furrows or constrictions appear at the periphery of the cytoplasm, midway between each nucleus, and spread inwards till they meet at the centre, cutting the four nuclei apart from each other; the larger spindle fibrils offer a certain resistance to the advance of the furrow, which appears to pass round them, but they eventually retract and take no further part in the cell processes. The hyaline layer already described closely follows up the advancing groove, flowing in from each side, but the adjacent surfaces do not fuse, and a definite partition line is left, along which separation eventually occurs (Plate VIII., Fig. 15, and Plate VI., Figs. 53-55).

After the separation of the pollen nuclei, a slight contraction of their cytoplasm takes place to form some six or eight indentations in the periphery (two or three visible in section), and at these points presently are formed an equal number of extranuclear bodies which are apparently concerned with the formation of the germ pores of the pollen-grain wall, since they are always found opposite these pores at a later stage; they are arranged evenly round the protoplast and increase but slowly in size. Their composition is obscure; they stain vigorously with Orange G, "Heidenhain", and "Delafield," but the colouration with the last two stains is considerably more brown than that of chromatin. At a later stage they appear to pass

* These can be seen more distinctly in Upland material.

from the cytoplasm into the pollen wall, where they apparently inhibit its growth in thickness above them, and they finally disappear at the same time as the hyaline plasma in which the wall is laid down.

A full account of the formation of the elaborate wall of the pollen grain is beyond the proper scope of this paper, but the following details are available—

After the separation of the tetrad nuclei, the hyaline layer round each increases considerably in width (Plate VI., Fig. 56), and a rather thick wall is differentiated at its inner surface. The spines of the wall, the perispore proper, are developed in the hyaline layer outside the thick wall, the mamelon on which each spine rests arising at the surface of the wall, though not till the spines have been completely formed. At the same time the wall itself is differentiated into three concentric zones; an exine or exospore, at the surface of which the mamelons are produced, a transparent mesospore, which later shows radial lamellation into "rodlets," and a denser endospore. There are also indications of a very thin limiting pellicle at the surface of the cytoplasm, which is for the most part not in contact with the developing wall (Fig. 58).

During the formation and differentiation of the wall, the pollen grain is not spherical, but collapsed into an irregular form, largely concave (Fig. 57). Since only a limited area of the cytoplasm is in contact with the wall, this may be regarded as a further example of the anomalous growth of spore walls separated from their protoplasts described by Beer³⁸ in the Onagraceae and by Tischler³⁹ in sterile hybrids of *Mirabilis*. There is, however, some diminution of the protoplast during the differentiation of the wall, pointing to a certain expenditure of protoplasmic substance in its production. As the spines of the wall are formed the hyaline layer disappears and can no longer be found when the process of wall differentiation is completed.

The tapetal layer remains intact until the hyaline layer is removed, when it breaks down to form a typical "plasmodium" which flows in among the grains, the protoplast of which at once begins to increase in volume, until the whole grain is filled with a dense and rather fibrous cytoplasm and its shape becomes spherical. Simultaneously with the irruption of the plasmodium the pollen wall becomes cutinised, taking on a yellowish colour and no longer staining with "Delafield." The pollen grain continues to increase in size until the anther bursts, the wall, now in contact with the protoplast except at the germ pores, increasing in area by intussusception without alteration in thickness, and shortly before the flower opens the nucleus divides again to form the generative and tube nuclei, the latter being slightly the larger of the two. No details of this division are at present available, though it appears that no perinuclear zone is formed in the process.

Thread Rings

The material on which Balls based his theory of the thread-ring mechanism of nuclear division was a commercial strain of *Mit Afifi*, an Egyptian cotton of the *G. barbadense* type; it is therefore not unreasonable to use the material of the present investigation for comparison. The main outline of this theory may be quoted briefly. As the spireme loosens out from synapsis, it shows as a continuous thread in which are embedded rows of granules, each showing a distinct longitudinal bisection.

"The nucleolus next decreases in size and the granules stain more darkly . . . the darkening is localised to those portions of the thread which correspond to the future position of the chromosomes. Except in these portions, the thread of the spireme is now split. Each of these clusters of darkened granules becomes . . . a bivalent

chromosome. This chromosome is not, however, merely bisected as were the granules, but is also divided transversely to the axis of the spireme thread; four perfectly distinct chromatic areas are thus formed, being the univalent chromosomes which are to be distributed to the four microspores . . . the chromosomes are bunched together at one side of the nucleus, and there is no peripheral distribution. In this respect cotton differs from most organisms and from its own vegetative cells. The two halves of the split spireme move apart from one another at the side where the chromosomes are lying, but this separation does not affect the chromosomes. The chromosomes are isolated by the removal of the spireme halves, but not entirely, for continuity with the latter is maintained by thin filaments on either side. The insertion of these filaments (the young spindle fibres) in the spireme halves causes slight swellings which appear to the eye as black dots.

From this point I propose to refer to the spireme halves as the "thread rings," retaining the term "fibre" for its usual subject.

The two thread rings continue to separate, the fibres becoming longer and longer, until the part of each thread ring which bears the dots has moved to a pole of the nucleus. The dots are scattered round some 100° of arc, and the remainder of each thread ring forms an irregular tangle of granular cytoplasm. The looping of thread rings seems to be due to the fact that the circumference of the close spireme is greater than the circumference of the nucleus. This stage constitutes the multipolar spindle.

The next event is the contraction of the dotted portions of the thread rings, bringing the dots nearer together and forming the bipolar spindle of metaphase. The looped thread rings lying in the clear zone between spindle and granular cytoplasm are quite conspicuous; this was the observation which initiated the present research. It sometimes happens that a stray dot is not drawn up into the cluster at the pole as soon as it should have been; in this case the spindle fibre which ends in it remains at the side of the spindle, often slack and bent, instead of being drawn taut.

The polar dots at the ends of the fibres have been noticed by other observers, but I can find no mention of their cross connection by the threads."

It has been shown in the present paper that the reduction division of the microspores of cotton proceeds along fairly normal telosynaptic lines, in agreement with the presentation of the facts first presented by Cannon (with a few exceptions of minor importance), and it is perhaps unnecessary to analyse all the points in which the account of Balls differs. The two main discrepancies (leaving out of question the method of conjugation) are the supposed bunching of the chromosomes at one side of the nucleus, prior to diakinesis, and the existence of the loops figured by Balls between the perinuclear zone and the spindle. While the former may perhaps be explained by reference to the second contraction of the spireme, which is not mentioned in Balls' account (although the passage in Cannon's paper to which he refers certainly describes it), the question of the loops within the perinuclear zone is more complex. Achromatic ring figures of a sort have undoubtedly been seen from time to time during diakinesis, metaphase, and anaphase by the writer, not only in cotton (Fig. 59) but in *Allium*, *Crepis* and *Dolichos*, but always in imperfectly fixed material, and no reference has been found to such rings in cytological literature.

Balls does not claim that the thread-ring mechanism is peculiar to the meiotic divisions, but states that it may be clearly seen in the somatic mitoses of the root tips. Several hundred sections of root tip material have been examined in an endeavour to observe thread rings, using various stains and fixatives, but with negative results, though all stages of somatic mitosis have been searched. In the somatic cells there is often a distinctly reticulate structure in the cytoplasm in which individual reticulations might by their size and prominence be taken for thread rings, though they may be seen in all parts of the cytoplasm at all stages of nuclear division, and particularly with the nucleus in the resting stage.

A large number of microspore heterotype and homotype spindles have been similarly examined under critical conditions in single and serial sections, with equal lack of success. Occasional well-marked rings have been seen,

usually at one side of the spindle (Plate VIII., Fig. 20). These might be explained as stray mantle fibres, but are more probably due to the fixation of the karyolymph in the form of a coarse reticulum. The nature of this reticulum and the fineness of its meshes varies according to the fixative employed. "Flemming" and "chromacetic" give a coarse network with some distortion of the spindle fibres, while with "Von Tellyesniczky" or "Bouin" the reticulation is almost invisible. In one slide a similar small ring occurring near the plate in metaphase proved to be visible in four successive sections, and was apparently a cylindrical or spherical inclusion of some kind. Small rings can also be seen in badly fixed multipolar stages where, under Balls' theory, they should not be visible (Plate VIII., Fig. 19). It is concluded that the appearances on which the thread-ring theory was based were due to imperfect fixation, under extremely trying working conditions, at a date when the full importance of the mode of chromosome conjugation had not yet been realised.

It must be pointed out, however, that the cotton plant, with its large number of minute chromosomes and its complex cytoplasmatic organisation, is by no means a suitable subject either for the establishment of new cytological theories or for criticism of existing hypotheses. The structures in question are barely within the limits of effective visibility and the possibility of subjective error is large.

Chromosome Numbers

The chromosome number of Sea Island cotton, *Gossypium barbadense* var. *maritima*, Watt, has not been previously determined. The type examined by Balls was commercial *Mil Afifi*, an Egyptian derivative of *G. barbadense* probably, though placed by Watt under *G. peruvianum*, Cav., and its chromosome number was stated by Balls as 20. Haploid material of *Mil Afifi* has not yet been available for examination, but a number of good chromosome plates have been found in transverse sections of the meristem of root tips from seed of the commercial cotton. The chromosomes in the somatic cells are in the form of short rods, generally curved, and resemble those of similar cells in the Sea Island types very closely. Counting is a matter of some difficulty, even under the highest magnification, but the number can be stated with certainty as greater than 50, and has been counted as 52 on several occasions: it would appear that the number given by Balls, which would be 40 in the somatic cells, is an under-estimate.

Cannon, working with F_1 hybrid material of *G. barbadense* (commercial Sea Island, Constellation brand) \times *G. hirsutum* (commercial Upland, Klondyke brand), found the chromosome number to be 28, though unfortunately neither of the parent types were studied cytologically.

Repeated counts of perfect plates in all the material examined show that the number of the chromosomes in all types of Sea Island cotton is more probably 26 than 28. The slight doubt arises from the heterogeneity of the chromosome size and shape, and it appears that there are two chromosomes markedly larger than the remainder, with a slight constriction which gives them the appearance of closely adjacent or "clumped" individuals. Similar constrictions have been recorded in chromosomes of *Vicia* and *Najas* by Sakamura^{40, 41}, and in *Fritillaria tenella* by S. Nawaschin,⁴² and their bearing on the apparent chromosome number and its variation in individuals of the same species has been discussed by several writers, including Sakamura, Winge⁴³ and Tischler.^{44, 45}

There are difficulties in the counting of large numbers, where it is impossible to check by reference to profile views, and errors may arise in several ways; from the counting of a plate which is still in late diakinesis, with some of the chromosomes clumped or at different levels, giving too small a number; from counting an early anaphase, where some of the chromosomes have already separated; from the presence of laggard chromosomes which have not taken up their proper position, or precocious chromosomes which pull away in advance of the others. In all cases the serial sections above and below should be examined, particularly with thicknesses of less than 5 μ .

It is not possible to arrive at chromosome numbers in doubtful cases by a statistical treatment of the counts, on account of the peculiar nature of the errors of observation, and the decision must always rest on personal judgement. At the same time, the tendency to give a "probable" number must be guarded against. Winge⁴⁴ has summarised this danger as follows—

"There is a general inherent inclination in the human mind, when dealing with numerical questions, to grasp at the "nice" figures. . . . The even numbers and highly divisible values have a certain attraction in themselves which is naturally increased when other considerations speak for their selection. Every cytologist is brought up to regard it as a fact that the chromosomes are halved before a division of the cell, and that the number of chromosomes is alternatively halved and doubled during the alternation of generations; there is therefore a natural temptation to find highly divisible multiples of 2 throughout. . . . A considerable number of incorrect chromosome values have indubitably resulted, as many plants, either through the presence of an especially great number of chromosomes or by the minimal size or awkward situation of the same, have offered particular difficulties in determination of the number, and thus led the investigator to decide the point, albeit perhaps unwittingly, according to his personal estimate."

It is often possible to check chromosome numbers by comparison of other species of the same genus. Winge⁴³, investigating the Chenopodiaceae, found the chromosome numbers of 11 species in 5 genera to be either 6, 9 or 18—i.e. 3×2 , 3×3 and $3 \times 3 \times 2$ respectively. In the Ranunculaceae the cardinal number appears to be 6; in *Crepis*, however, the cardinal number may be 3, 4 or 5, though Miss Digby¹⁴ quotes the observations of Fraser and Snell⁴⁶ on constrictions as a possible explanation of the anomaly.

Unfortunately, the only chromosome numbers of the Malvaceae yet published are the two rather doubtful values for *Gossypium* (20 and 28) already quoted. These are both easily divisible multiples, and more attractive than 26, with its cardinal number of 13 as against their 5 and 7. Until further plates are available in other species of *Gossypium* and other genera of the Malvaceae it is evident that the comparative method is useless; at the same time it may be pointed out that there is no inherent improbability in the number 26, for in Tischler's⁴⁵ latest list a number of plants may be found with the cardinal number 13, and in the Aceraceae recently investigated by Taylor²⁹, three species have 26 chromosomes and two 13, although the latter does not appear to be the cardinal number for the genus.

Abnormalities

In the first-generation hybrid investigated, Cannon found a number of abnormalities, chiefly of the nature of amitotic divisions. These were not observed by him in greenhouse material collected in November and December, but occurred in material taken in the spring. Cannon expresses a doubt "whether these abnormal divisions were due to cultural conditions, to the fact that the plant was a hybrid, or to both these factors with the added one that the flowers were the last to form on the plants." Balls states that these

irregular divisions can be found in all cotton plants if very late flowers or very early ones on ratoons are taken, and that they are not necessarily due to hybrid constitution, but might be provoked by the greenhouse culture employed.

In the very large number of buds taken for the present investigations at all times of the year and from plants in all stages of growth, including ratoons, there is no evidence whatever of amitotic division, either in the pure Sea Island strains or in commercial Upland varieties. This would point to some other cause than greenhouse culture for amitosis; bad culture, with extremes of temperature and widely varying humidities, tends to produce considerable bud shedding, but in these circumstances a general necrosis of the staminal column ensues, clearly recognisable in its earliest stages. It is conceivable that in the cases described by Cannon the amitosis was due to the presence of a lethal combination of genetic factors acting on the microspocyte, though confirmation of this is lacking.

Such degeneration as was found in the material examined was of the nature of a localised contabescence, the contents of one or more loculi, in an otherwise normal anther, showing complete disorganisation. The nucleus breaks down and the chromatin is dispersed through the cytoplasm, which stains so deeply as to obscure all evidence of structure in many instances. Partly degenerate cells may show the nuclear wall still intact, but the chromatin irregularly dispersed in a coarse reticulum within it, while at the same time the cytoplasm contains chromidia-like bodies in large numbers throughout its substance.

This contabescence is still under investigation, but it appears that the normal secretion products of the cotton plant which occur in all the flower buds in isolated cells and lysigenous glands, may, under certain conditions, be secreted in the spore protoplasts, where they interfere with normal development and cause abortion. Evidence of similar secretion has been found in aborted cells of the megaspore; for fuller details of these substances and their occurrence in all parts of the plant and in other species of *Malvaceae*, reference may be made to the work of Stanford and Viehover.⁴⁷

In addition to contabescence, it is noteworthy that in two instances an apparent diploidy has been observed in the first meiotic division, though it was not possible in either case to count the chromosomes seen in profile, and it is not known how the duplication arose. In one case the nucleus was in late anaphase, but the shape of the chromosomes was typically meiotic; in the second the chromosomes were in late diakinesis, but in both the amount of chromatic substance was apparently double the normal, and the nuclei were abnormally large. No evidence of a similar condition has been observed with any certainty at any other stage, however, and all the other nuclei in these two buds were normal.

SUMMARY

The process of pollen formation in Sea Island cotton is described and figured.

The reduction division takes place along normal telosynaptic lines, and agrees on the whole with the process as described by Cannon in hybrid cotton plants.

Points of special interest occur (a) In the perinuclear zone, which is apparently largely concerned with spindle formation in both homotype and

heterotype divisions, but not in the premeiotic or somatic divisions; (b) in the manner in which the chromosomes are brought to the centre of the nucleus in diakinesis; and (c) in the method of tetrad division by furrowing.

The chromosome number is 26, with two chromosomes distinctly larger than the remainder.

Thread ring structures which have been described in parallel material have not been found in well fixed buds, but ring figures of a similar appearance occur in cells which show distortion by the fixative.

The writer wishes to record his thanks to Mr. H. Gunnery, who prepared most of the slides used and gave, in addition, much very valuable assistance in the working out of the cytological history.

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EXPLANATION OF PLATES I.—VIII.

Plates I.—VI.

All the figures were drawn with Zeiss large *camera lucida* under a $1/15''$ Koristka semi-apochromatic immersion objective, N.A. 1.30, with Zeiss compensating ocular 18 (old notation), and Leitz aplanatic condenser of N.A. 1.40 immersed, except Figs. 34-43, 46-52, 58, for which Zeiss 2 mm. apochromatic objective, N.A. 1.40, was used, and Figs. 53-57 with Zeiss 4 mm. apochromatic objective N.A. 0.95.

FIG. 1—Premeiotic division. Resting stage, large nucleolus (vacuolate) in achromatin bride across nucleus.

FIG. 2—Increase of chromatin by budding from nucleolus.

FIG. 3—Nucleolus disappearing; continuous spireme.

FIG. 4—Spireme thickens and fragments into chromosome lengths.

FIG. 5—Metaphase of premeiotic division.

FIG. 6—Early anaphase, chromosomes rod-shaped.

FIG. 7—Commencement of telophase, with aggregation of chromosomes.

FIG. 8—Telophase. The nuclear membrane reappears and cell-plate is formed.

FIGS. 9, 10—Segregation of chromatin into granules on continuous achromatin spireme. Two karyosomes present.

FIG. 11—The karyosomes have fused into one large vacuolate nucleolus, and the nucleus appears empty except for a few chromatin granules at the periphery.

FIG. 12—The nucleus increases rapidly in size; the achromatin reticulum spreads through the cavity, with chromatin beads at the intersection of the strands. Nucleolus markedly alveolate.

FIG. 13—The achromatin reticulum increases and thickens.

FIG. 14—The reticulum is now apparently double in parts.

FIG. 15—Commencement of synaptic contraction. The reticulum pulls away from the nuclear wall. Nucleolus less alveolate but still showing vacuoles.

FIG. 16—Close synapsis in its earliest stage.

FIG. 17—Synapsis. No trace of structure in the knot now visible. The nucleolus stains solidly.

FIGS. 18, 19—The synaptic knot loosens out and the spireme can be seen as double in a few places.

FIG. 20—The knot completely unravelled. The spireme is markedly double and chromomeres can be seen, with beads of chromatin at intersections.

FIG. 21—Commencement of open spireme; chromomeres clearly marked.

FIG. 22—Open spireme. Section below nucleolus. Some doubling of threads visible.

FIG. 23—Anastomoses disappearing. Much apparent doubling and chromomere lumps on thread.

FIG. 24—Completion of open spireme, no strands left in centre of nucleus.

FIG. 25—Commencement of second contraction, with marked thickening of spireme.

FIG. 26—Height of second contraction.

FIG. 27—Loosening out of thickened spireme, clearly double and twisted.

FIG. 28—Section through looped thickened spireme.

FIG. 29—Spireme segmenting into lengths.

FIG. 30—Condensation of spireme lengths to bivalent chromosomes. The nucleolus begins to disappear.

FIG. 31—Nucleolus almost gone. Clearly marked chromosome loops.

FIG. 32—The cytoplasm has taken on the typical radial structure, and the perinuclear zone has formed. The chromosomes are united by fibrils in this zone.

FIG. 33—The same stage as 32, a tangential section through the perinuclear zone showing fibrillar strands uniting all chromosome masses.

FIG. 34—The chromosome masses pass inwards to the centre of the nucleus, as the fibrillar band contracts, drawing the spindle fibres after them.

FIG. 35—Multipolar spindle from badly fixed material, with distorted spindle fibres and "rings."

FIG. 36—As the spindle fibres move round to the poles a tripolar spindle forms.

FIG. 37—Slightly later. A quadripolar spindle.

FIG. 38—Heterotypic metaphase. Some of the chromosomes beginning to pull out.

FIG. 39—Early anaphase; curved spindle with precocious chromosomes.

FIG. 40—Early anaphase.

FIG. 41—Anaphase with one pair of chromosomes drawn out into rods.

FIGS. 42, 43—Plates showing 26 chromosomes, united by faintly visible connections. In each case two chromosomes are markedly larger than the others.

FIG. 44—From two spindles in early anaphase. The chromatin is drawn out by the spindle fibres into irregular masses, suggesting the separation of the homotype chromosomes.

FIG. 45—Late anaphase.

FIG. 46—Early telophase. The chromatin is precipitated as granules on a very fine continuous spireme.

FIG. 47—The chromatin flows into a coarse reticulum (only instance observed; possibly abnormal intercalated resting stage).

FIG. 48—Typical interstage. The chromatin forms rounded masses slung on achromatin strands in the direction of the previous spindle fibres. A new perinuclear zone forms round each nucleus.

FIG. 49—An interstage. A faintly staining karyosome is present.

FIG. 50—A homotype prophase.

FIG. 51—Homotype metaphase. The spindle fibres of the heterotype division can still be seen, and the remains of the original perinuclear zone.

FIG. 52—Two homotype plates. That on the left is slightly oblique; the chromosomes are passing into anaphase. The right-hand plate is in late diakinesis.

FIG. 53—Division by furrowing. The furrow is seen commencing at the interstage between the first and second divisions. The outermost (continuous) line marks the edge of the hyaline plasma, the dotted line a faintly differentiated zone at the edge of the cytoplasm.

FIG. 54—Pollen tetrad in tetrahedral arrangement. Furrowing has commenced at the edges and has already reached the centre from below.

FIG. 55—Pollen tetrad in flat plate. An advanced state of furrowing.

FIG. 56—The tetrad completely separated. A dense wall is differentiated at the edge of the cytoplasm and a number of deeply staining bodies have appeared in each cell.

FIG. 57—Formation of pollen grain wall in collapsed state. Hyaline plasma not shown.

FIG. 58—Section through the wall of mature pollen grain at a germ pore. The limiting pellicle is shown black.

FIG. 59—"Rings" due to faulty fixations; an early meiotic anaphase.

PLATES VII. and VIII.

All the figures are from microphotographs taken with Zeiss 4 mm. apochromatic objective N.A. 0.95, Leitz applanatic condenser N.A. 1.00 dry (3.4 cone). Wratten filters 45 and 62. Ilford ordinary plates. Figs. 4, 5, 6 and 7 taken with Leitz periplanatic eyepiece 6 \times , remainder with periplanatic 10 \times .

FIG. 1—Presynapsis. The upper cell shows the reticulum just pulling away from the nuclear wall; in the lower cell the contraction is well advanced.

FIG. 2—Synapsis.

FIG. 3—Loosening out from synapsis.

FIGS. 4, 5—Later stages of loosening out.

FIGS. 6, 7—Nucleus passing into open spireme.

FIGS. 8, 9—Open spireme with gradual thickening of thread.

FIG. 10—Second contraction.

FIG. 11—Thick spireme loosening out, with twisting.

FIG. 12—Spireme commencing to segment.

FIG. 13—Segmentation. Perinuclear zone well marked, and cytoplasm showing coarse radial reticulation.

FIG. 14—Perinuclear zone and fine radial reticulation.

FIG. 15—Concentric fibrils uniting chromosome masses. First appearance of hyaline zone.

FIG. 16—Chromosome masses passing in towards centre.

FIG. 17—Multipolar spindle.

FIG. 18—Tripolar spindle.

FIG. 19—Two multipolar spindles from badly fixed material, showing distorted fibres and "rings."

FIG. 20—Heterotype spindle, slightly distorted. Note ring at lower end and others at extreme right of cytoplasm.

FIG. 21—Heterotype plate.

FIG. 22—Anaphase.

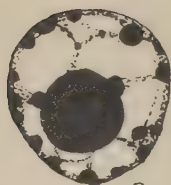
FIG. 23—Interstage. Hyaline zone well marked.

FIG. 24—Homotype division; chromosomes being drawn into centre of nucleus by contraction of fibrillar ring.

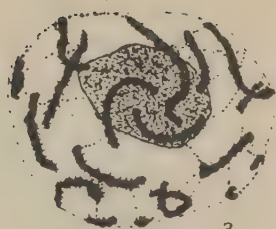
FIG. 25—Division of tetrahedral tetrad by furrowing.



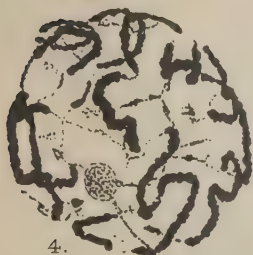
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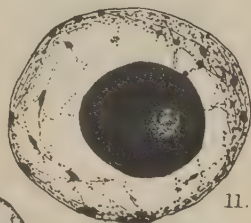
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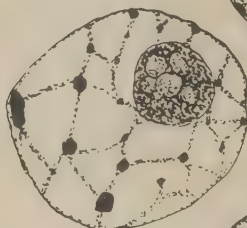
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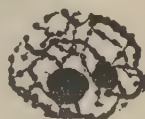
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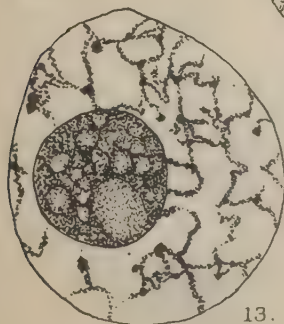
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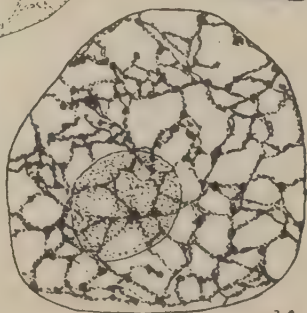
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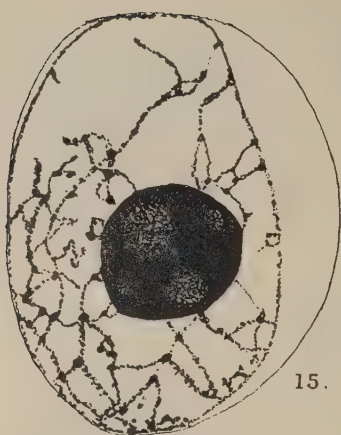
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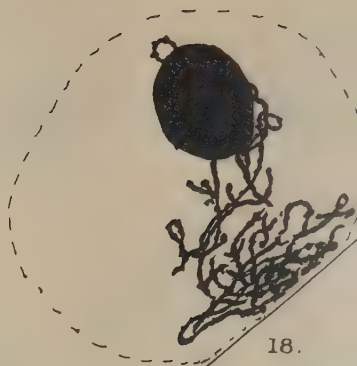
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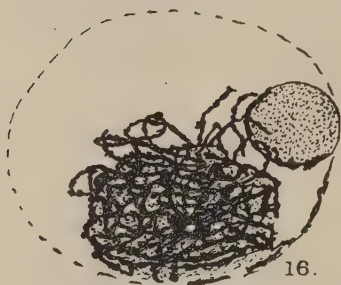
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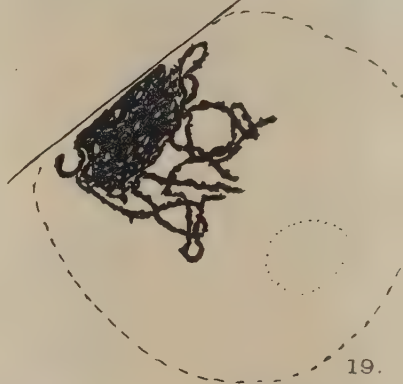
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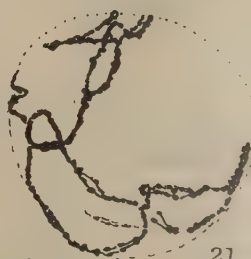
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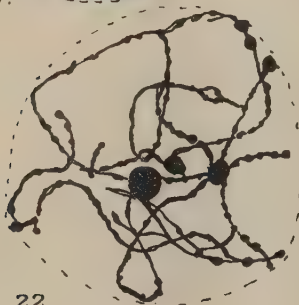
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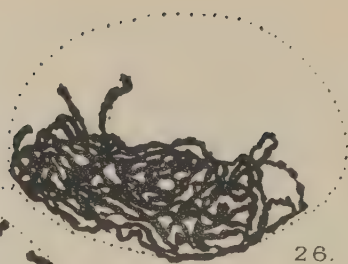
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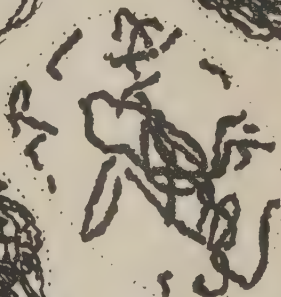
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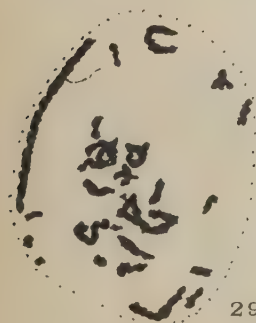
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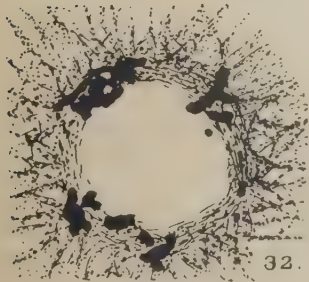
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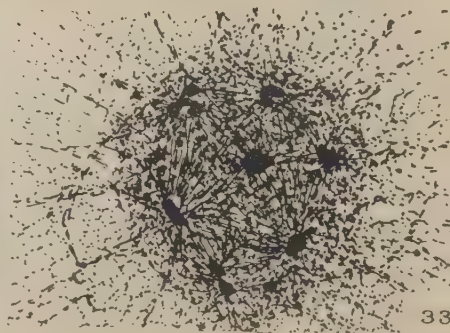
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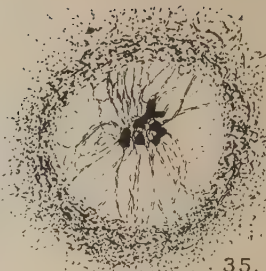
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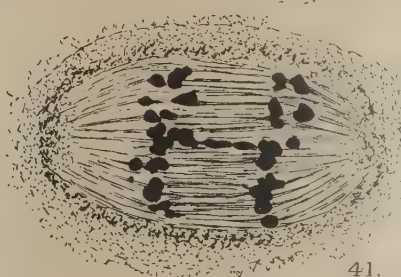
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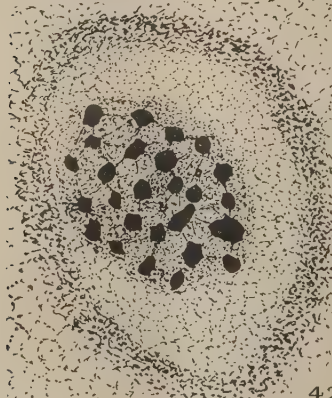
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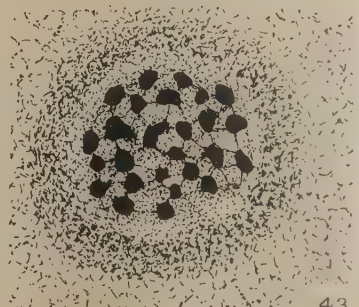
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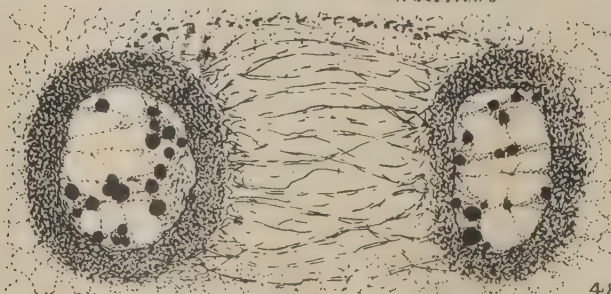
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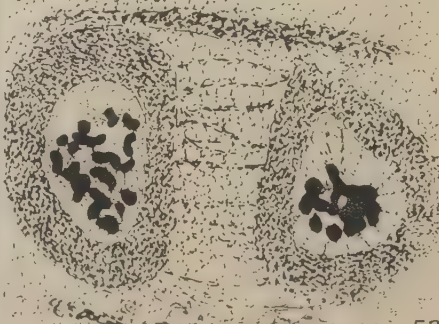
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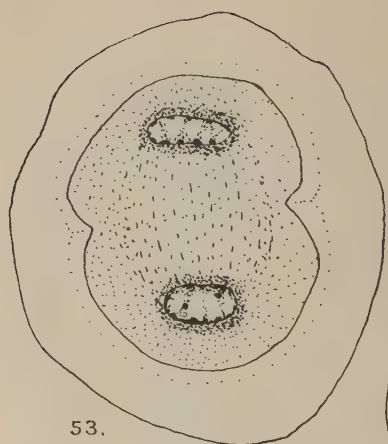
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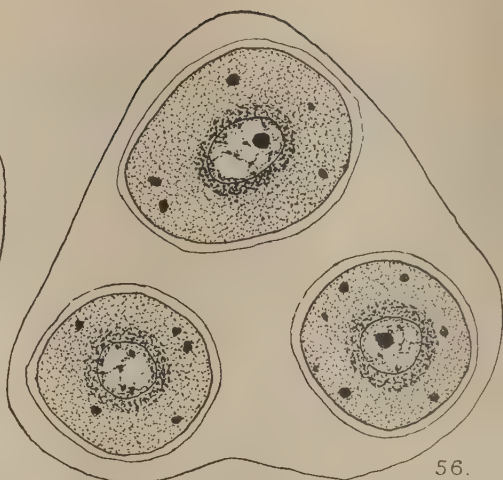
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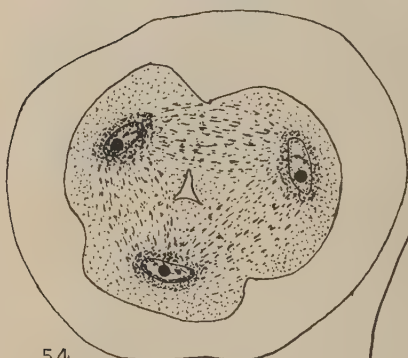
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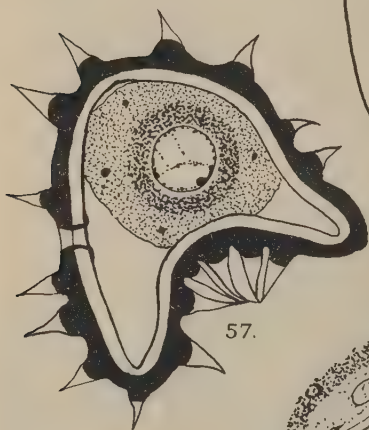
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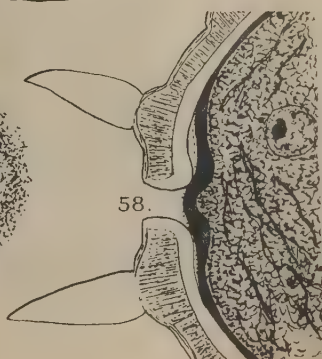
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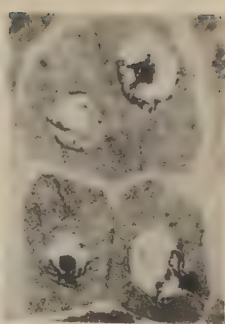
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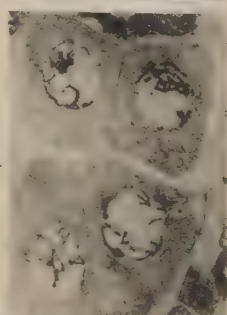
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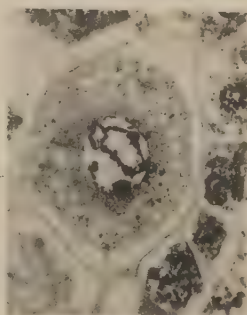
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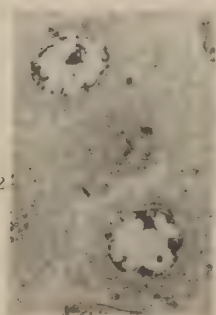
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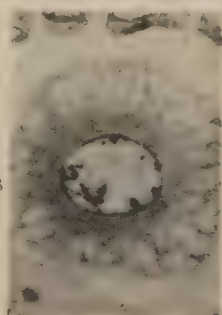
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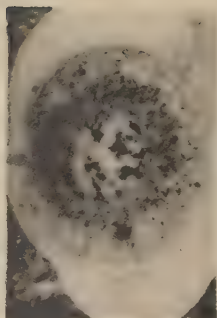
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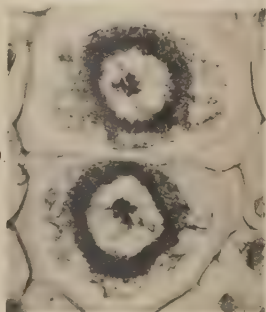
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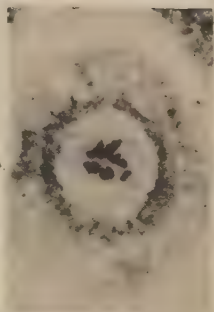
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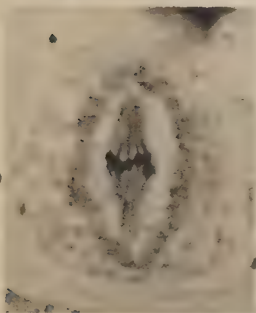
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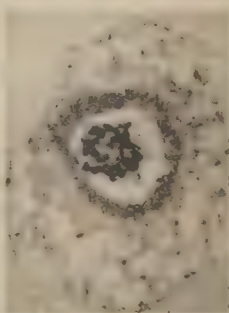
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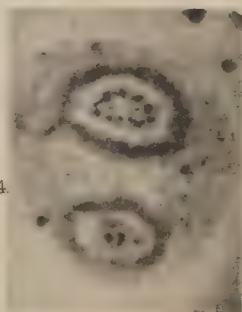
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XXI.—THE CYTOLOGY OF THE COTTON PLANT

ii.—CHROMOSOME NUMBERS OF OLD AND NEW WORLD COTTONS

By HUMPHREY JOHN DENHAM, M.A.(OXON), F.R.M.S.

(The British Cotton Industry Research Association.)

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INTRODUCTION

In the first paper, the cytology of pollen formation in Sea Island cotton (*Gossypium barbadense*, var. *maritima*, Watt) was discussed in some detail. The writer is now in a position to give the chromosome numbers of the several types which have been cultivated in the experimental greenhouse of the Shirley Institute at Didsbury in the last three years.

The chromosome number of Sea Island cotton has already been given as 26. It has now been found that Upland types give the same number as Sea Island in every case, but that Indian and Chinese cottons so far examined have 13 as their chromosome number. Detailed lists of the types examined cytologically are presented, with figures of chromosome plates of some of the main varieties; all the numbers should be read as haploid, unless definitely stated as diploid.

MATERIAL AND METHODS

The methods used are substantially as described in the previous paper. Normal Bouin's fixative was used in most cases (picric acid, sat. aqueous sol., 75 parts; commercial formalin, 25 parts; glacial acetic acid, 5 parts), as it gives freedom from "clumping" of chromosomes, at the expense of slight distortion of finer cytoplasmatic details. The fixative recommended by Karl Sax¹, a modified Bouin's solution developed by Allen² and containing chromic acid and urea, was tried but found to contain incompatible substances giving rise to violent effervescence and a muddy precipitate on mixing. The chromosomes were counted with objectives of high resolution both directly and with an Abbe drawing apparatus. In most cases the numbers were checked by several independent observers (S. C. H., G. G. C., D. A. and H. G.). Drawings (Figs. 1-11) were made with Zeiss 2 mm. homo. mm. objective, Zeiss K.18 ocular (new series), and Zeiss large Abbe drawing apparatus, at a magnification of 2,400, reduced in printing to 1,500. Heidenhain's haematoxylin was used in every case. It has not been found

practicable to identify the individual chromosomes, which are too minute for the purpose; nor can too much reliance be based on the actual size of the chromosomes, owing to a well-known physiological error introduced in the drawing of small dark bodies on a background of variable intensity. The material was grown from seeds derived from the several sources mentioned. It is noteworthy that whilst no difficulty has been found in getting plants of Sea Island, Upland and Indian cottons to flower in the greenhouse at all seasons of the year (flowers are self-pollinated and all seed produced is viable), no such success has been obtained with Trinidad Native (tree types) and *Gossypium brasiliense*, while Egyptian cottons are persuaded with difficulty to produce buds, and no cytological material has been obtainable which shows haploid plates.

TYPES EXAMINED

Sea Island Cottons

Gossypium barbadense, var. *maritima*, Watt. From seed of Dr. Harland's pedigreed types, brought from St. Vincent.

V. 135.	B. D. 9-6.	A. E. 17-5-6.
V. 74.	U. S. 2.	A. R. 3-19.
D. 134.	G. X. 12-2-9-19.	A. N. 28-19. 13-56.
H. 23-5-21.		A. K. 41-18.

These are the plants from which the material for the previous paper was taken and agree in having a chromosome number of 26. Two chromosomes are noticeably larger than the remainder.

American Cottons

These are grouped by Watt³ as falling for the most part under *G. hirsutum*, Linn., and *G. mexicanum*, Tod.

<i>Acala</i> .	Seed from U.S. Dept. of Agriculture. A large-bolled Upland variety of <i>G. mexicanum</i> type	26
<i>Commercial—I</i> .	Flowered in greenhouse of Botanical Dept., University of Manchester. A commercial cotton of unknown ancestry; small boll, hirsute	26
<i>Commercial—II</i> .	Seed from a plant given by Mr. W. Greenwood, M.P.; a large-bolled, semi-hirsute type, near <i>G. hirsutum</i>	26
<i>Indian American</i> .	289 F. Seed of this and the following variety was sent from Cawnpore by Dr. Martin Leake; a small leaf, medium-sized boll, hirsute	26
<i>Indian American</i> .	285 F. A similar plant to 289 F. No details of their ancestry at present available	26

Egyptian Cottons

These appear more closely related to *G. barbadense* than to *G. peruvianum*, under which they are placed by Watt.

<i>Mit Afifi</i> .	Commercial. Seed given by Dr. W. L. Balls; flowered with difficulty, but no haploid material available. Chromosomes counted in root-tip. Diploid number	circa 52
<i>Giza—I</i> .	This and the following are two distinct types, from seed sent by Cotton Research Board, Egypt	26
<i>Giza—II</i> .	Could only be counted in root tip. Diploid number	circa 52
<i>Pima</i> .	An American-Egyptian of mixed parentage. (See Kearney ⁴ ; probably including commercial Upland and "Hindi Weed." No perfect plates	circa 26



FIGS. I-II. Chromosomes of various types of Cotton. 1. Acala. 2. Indian American, 289f. 3. American Commercial I. 4. American Commercial II. 5. Chinese naked seeded. 6. *G. cernuum* \times *rudicum*. 7. *G. arboreum* \times *neglectum*. 8. *G. roseum*. 9. *G. neglectum*. 10. *G. arboreum*. 11. *G. sanguineum* \times 1500.

Indian and Chinese Cottons

Seed in every case sent from Cawnpore by Dr. Martin Leake. Chromosome number 13, with one chromosome larger than others.

<i>G. sanguineum</i> , 27, Tod; placed by Watt as <i>G. arboreum</i> , Linn., var. <i>sanguinea</i> , Watt	13
<i>G. sanguineum</i> , 124	13
<i>G. roseum</i> , Tod= <i>G. arboreum</i> , Linn., var. <i>rosea</i> , Watt	13
<i>G. arboreum</i> , Linn.	13
<i>G. neglectum</i> , Tod= <i>G. arboreum</i> , Linn., var. <i>neglecta</i> , Watt	13
<i>G. arboreum</i> × <i>neglectum</i> , Leake	13
<i>G. cernuum</i> , Tod= <i>G. arboreum</i> , Linn., var. <i>assamica</i> , Watt	13
<i>G. indicum</i>	13
<i>G. cernuum</i> × <i>indicum</i> , Leake	13
<i>G. mollisoni</i>	13
Chinese naked seeded. Possibly a form of <i>G. Nanking</i> , Meyen?	13

Other Species

Columbian Native. From seed collected in interior of Colombo by Mr. R. Mordecai. Possibly either *G. mustelinum*, Miers, or *G. peruvianum*, Cav., but not yet grown; from root tip. Diploid number *circa* 52

The above list is necessarily incomplete, and it is hoped that workers in other parts of the world will be able to record further numbers of this interesting genus in the near future. The types particularly needing elucidation still are the Mediterranean *G. herbaceum*, Linn., and the many other varieties which have been placed under this name; "Hindi Weed" and the tree cottons of the Sudan; *G. peruvianum*, *brasiliense*, *mexicanum*, *vitifolium*, and many other parents of commercial strains; while the almost hairless aboriginal types, such as *G. Sturtii* or *Sturtia* of Australia and *G. Stocksii* of India, are of great botanical interest. The relations of other genera of the Hibisceae are still questionable, especially the nearly related *Thurberia*, *Fugosia* and *Thespesia*; whilst there are singularly few numbers known in the whole order Malvaceae, where there is reason to believe that cytological peculiarities abound.

The abrupt separation of the genus *Gossypium* into two groups in which the chromosome number of the one is twice that of the other is in itself a matter of great interest. It is known that interspecific hybrids can be made with comparative ease in either of the groups, but as far as can be ascertained, no plant-breeder has yet been successful in crossing an American or Egyptian cotton with a true Indian cotton (it has been stated that this cross has been made in Russia, but no reference can be found to it in the available literature, and it is probable that the cross has now been lost). At the same time, it may be observed that the plants of the 26 group are, on the whole, much larger than those in the 13 group; the stems are taller, the leaves, flowers and bolls are larger, and the lint is longer. It is a matter of speculation whether this is a case of "gigantism" due to the double chromosome number, though comparable phenomena have arisen in the mutants of *Oenothera* and other species. The fact of the difference may perhaps open a further possibility for the plant-breeder who wishes to make the cross between Indian and American cottons; if it can be assumed that there is any hope of finding a diploid mutant of an Indian cotton (which would

probably reveal itself as suitable for cytological examination by its abnormal size) it might reasonably be expected to prove a fertile parent for the experiment.

Until further chromosome numbers are available, it is inadvisable to draw any conclusions as regards the systematy of the genus, which is still in a far from satisfactory state, in spite of the work of Todaro⁵, Parlatore⁶ and Watt; the question of the identity of *G. hirsutum*, which is described by the latter as common to Europe, Asia, Africa and America, may be cited as an example. At the same time it is possible that the new evidence obtainable from the chromosome numbers will settle the debate as to the origin of cotton in America. The earlier explorers all stated positively that they found cotton growing and in use⁷. Wiener⁸, however, has recently made the startling contention that what they saw was not *Gossypium*, but *Bombax Ceiba*, and that cotton was introduced from Europe by Columbus and his followers, or from Africa by the negro overseers of the early plantations—notwithstanding the evidence of the Peruvian tombs.

SUMMARY

The chromosome numbers have been counted in some thirty-two varieties of cotton, including American, Sea Island, Egyptian, Indian and Chinese types, and American grown in India. These numbers fall into two groups of 26 and 13 chromosomes, the former comprising the cottons of the New World and Egypt, and the latter those of Asia. This may, perhaps, explain the impossibility of crossing American or Egyptian cottons with Indian types, and suggests a possible solution of the difficulty with the help of cytology.

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